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BIOLOGICAL PROBLEMS in WATER POLLUTION

Third Seminar

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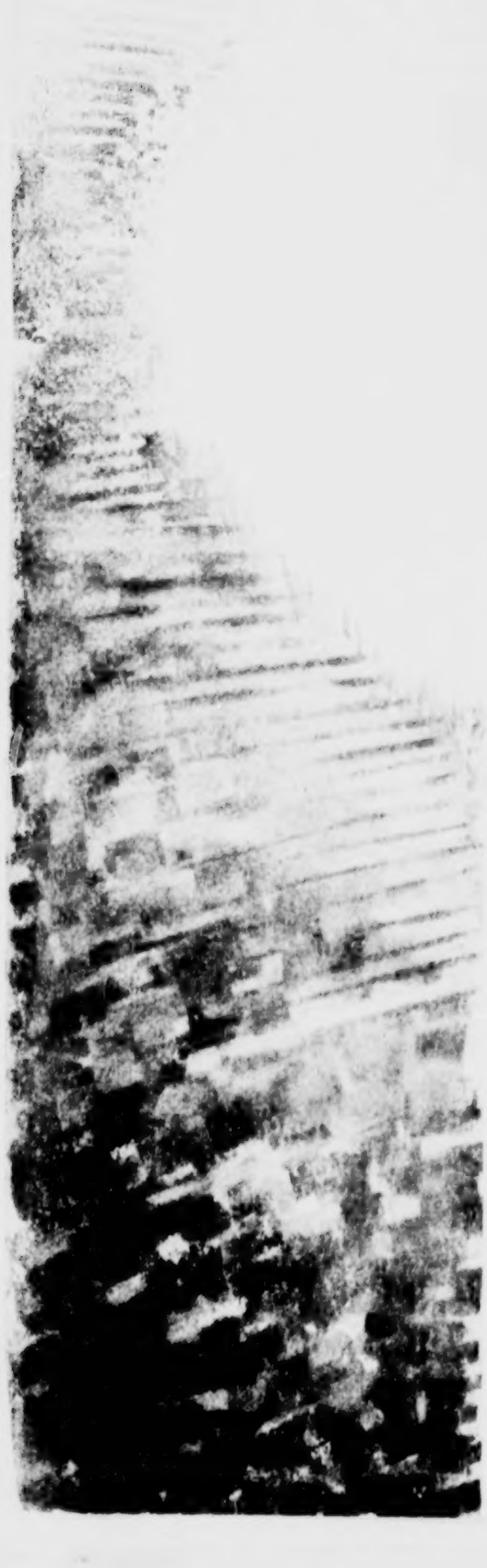

BIOLOGICAL PROBLEMS IN WATER POLLUTION
Third Seminar
1962

Planned and Assembled
by
Dr. Clarence M. Tarzwell
Chief, Aquatic Biology

Research Branch

Robert A. Taft Sanitary Engineering Center

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Division of Water Supply and Pollution Control
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1965

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FOREWORD

The Seminars on Biological Problems in Water Pollution were initiated in 1956 and have been continued because it is believed they meet a need in the young but growing field of sanitary biology.

The objectives of the seminars are:

1. To bring together those engaged in biological research and investigations of water pollution problems so that they may become acquainted with each other.

2. To learn of current developments in this field, research and investigations in progress, and the most recent findings and problems.

3. To carry on discussions that will indicate new approaches to old problems, define new problems, iron out disagreements as to approaches and basic philosophy, generate new ideas and new perspectives, and assist in the solution of common or troublesome problems.

4. To acquaint those in the field with new equipment, experimental procedures, and methods of handling, analyzing, and evaluating data so that they may better approach the problem.

5. To enable those engaged in training future workers to learn first hand what those who are presently engaged in the field believe should be included in the curricula of training programs.

6. To broaden viewpoints, assist workers in comprehending the overall problem, and, above all, to stimulate them and create a greater incentive and appreciation for their work and a keener understanding of their role in this public service.

These seminars have been set up as discussion meetings with emphasis on participation by those in attendance. Sessions are a half day or a day in length and each deals with a particular subject. Papers for each of the sessions are by invitation and each paper deals with a particular phase of the subject, which thus is covered in the most adequate manner. The number and length of papers are limited to provide ample time for discussion from the floor.

In addition to the more formal seminar, informal sessions on pertinent topics are scheduled. In these informal sessions, only one paper is presented by the discussion leader; the rest of the session is devoted to questions and a thorough discussion of the subject. These informal sessions have proven to be very popular and many believe they are the most important part of the seminar.

Plans for the third seminar were begun shortly after completion of the second seminar in 1959. In viewing items of current importance and interest, it appeared that water supplies of adequate quality and quantity were or would shortly be of prime importance. It soon became evident that with a fixed fresh water supply the best method of augmenting this supply was to use the same water over and over. Such reuse, however, requires that each user return the water to its source in a condition satisfactory for another use. To do this effectively and efficiently, he must know the quality of water required for the desired use. In the case of the production of aquatic life, we must know the quality of water required for the survival, growth, reproduction, general well being, and the development of an adequate crop of the desired organisms. In short, we must have water quality criteria for the protection of aquatic life. It was judged, therefore, that a seminar on water quality criteria for aquatic life would be both timely and beneficial. Such a seminar would serve to assemble, present, and evaluate current information on the environmental requirements of aquatic organisms, which would lead to sounder conclusions on which to base water quality criteria for their protection. It was hoped that the transactions of such a seminar would serve, at least initially, as a handbook in the field and a stimulus for further work essential for the setting of better criteria.

The seminar was timed for the week preceding the 14th meeting of the International Association of Theoretical and Applied Limnology held in August 1962 at Madison, Wisconsin. This enabled foreign scientists to attend and contribute to both meetings. In formulating the program for the third seminar, a world-wide search was made for people knowledgeable in the environmental requirements of certain organisms or groups of organisms. That this effort was fruitful is attested by the roster of participants.

Those in attendance from outside the United States numbered 88, in a total registration of 436, and represented 26 countries. Canada led in the number of delegates with representatives from all the provinces; several other countries were well represented. In the United States, 43 states and the District of Columbia were represented. Universities contributed the largest single group, with conservation agencies ranking second. Agencies and groups represented were the U.S. Public Health Service, U.S. Fish and Wildlife Service, U.S. Department of Agriculture, Tennessee Valley Authority, Atomic Energy Commission, Bureau of Reclamation, Great Lakes Fishery Commission, Ohio River Valley Water Sanitation Commission, Food and Agriculture Organization, National Wildlife Federation, Sport Fishing Institute, Audubon Society, Conservation Foundation, and Federal, State, and Provincial Conservation, Health, and Water Pollution Control Agencies.

The deliberations, presentations, and discussions of this group are presented in these transactions. Every effort has been made to present all ideas expressed during the discussions in both the formal and informal sessions. It is hoped that these transactions will prove of real value not only to those who attended, but to workers in the field of water pollution everywhere.

Clarence M. Tarzwell

ACKNOWLEDGMENTS

The translations of the abstracts of the papers in these transactions into French and German have been made possible through the kind assistance of a number of people. Dr. Paul Rothemund, Chemist, of the Aquatic Biology Section, Basic and Applied Sciences Branch, Division of Water Supply and Pollution Control of the United States Public Health Service at the Robert A. Taft Sanitary Engineering Center translated the abstracts into German. We are indebted to the Fisheries Research Board of Canada through the efforts of Dr. John Sprague of their laboratory at St. Andrews, New Brunswick, for the translations into French of sixteen abstracts, including thirteen by authors from the United Kingdom. Miss Maud Nisbet of the Central Station of Applied Hydrobiology, Paris, France, kindly translated fifteen of the abstracts into French. We are also indebted to Mr. Robert J. Griffin, Jr., Administrative Assistant of Radiological Health Research Activities, Robert A. Taft Sanitary Engineering Center, for his assistance in the preparation of drafts and proof reading of many of the French translations. Mr. Griffin was also instrumental in securing the services of Mr. Alain Guillerd of Lyon, France, who translated the remainder of the abstracts into French.

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THE ROLE OF THE AQUATIC BIOLOGIST IN THE FEDERAL WATER POLLUTION CONTROL PROGRAM

James M. Quigley *

I am a lawyer by profession, a politician by nature, and an administrator by appointment.

In the light of this background, one might very well ask what then am I doing here addressing a scientific seminar on the subject of "Water Quality Criteria for Aquatic Life." The answer rests with my third "qualification." I am here, first, because I was appointed Assistant Secretary of Health, Education, and Welfare by President Kennedy, and second, because I was appointed by the Secretary of Health, Education, and Welfare to oversee the administration of our Department's Water Pollution Control Program.

To those of you who at this moment are experiencing an instinctive reaction something along the line of, "Why would anybody put a lawyer and a politician in charge of a Water Pollution Control Program?", all I ask is that you reserve final judgment until you hear me through. I ask this because I believe that, whether they realized it or not and whether you know it or not, the people who made such an appointment had you in mind when they made it.

Not you, as individuals. Not you, as aquatic biologists. But rather, you, as representatives of many individuals and many groups who have long had a deep and abiding interest in the ever-growing problem of water pollution in this country, and who have often experienced frustration in your efforts to make a more substantial contribution towards a solution of this national problem.

The aquatic biologist has a contribution to make to the solution of the water pollution problems of this country. If I tell you that I conceive it as one of my prime duties to see to it that aquatic biologists are given a greater opportunity to make such a contribution, you may just begin to appreciate why it was that this lawyer and politician was given the administrative assignment that has brought me here this morning.

For too long, problems of water pollution in this country have been approached in much too narrow, much too parochial ways. In a less complex society than we now live in the matter of water pollution invariably became a community issue only when it became a health hazard. Only when water made people sick did it become a matter of serious public concern. Nature has been abundantly generous to our America and, as a result, in the past in most areas of our country, when the fish stopped biting or the water tasted a little unpleasant, there was always plenty of other water to fish in or swim in or drink. Only when their lives or health were endangered were people ready to demand and support government action to abate pollution. Such government action usually involved the local Board of Health. Thus, the traditional pattern was established whereby water pollution was viewed as merely a local health problem, one that could and should be handled locally by doctors. Initially this may have been an accurate appraisal of the problem and under the circumstances more than often may have provided an adequate solution. Unfortunately, in many areas of the country, as our population exploded, our cities grew, our industries expanded, water pollution problems became something other than just a local and just a health problem. Even more unfortunate is that even to this day the realization of this development seems to have escaped the grasp of too many public health officials.

You in this audience know better than anyone else that water that may be fit to drink in the sense that it will not perceptibly harm human health may in fact be water that will not sustain fish life. In the traditional view of the public health doctor, this water would not be polluted. In your view as modern aquatic biologists, it surely is.

But I would be in error and unfair if I were to give the impression that all of the unsolved problems in the field of water pollution control should be laid on the door step of shortsighted public health doctors. While doctors may have been the dominant force, they have not and are not the only people involved in water pollution matters.

Engineers have been part of the water pollution picture for many years. Many of the long-proven effective sewage disposal techniques are the handiwork and the brainchild of sanitary engineers. For this, we can be and are grateful. But as one looks at the current state of the art of water pollution control one cannot help feel that too often sanitary engineers have been content to stay with proven old ways, to follow the beaten path. The boldness and imagination that have characterized engineering efforts in such varied fields as rocketry and saline-water conversion have all too often been lacking in their approach to water pollution control.

In my judgment, we have reached the point where we can no longer afford halfway measures, when we can no longer be satisfied with old-fashioned remedies however wonderful they were in grandfather's day. The nature and the scope and the volume of water pollution today is such that it challenges the engineering genius of our nation. There is need for extensive engineering research in this area. But it should not involve the mere expenditure of more money, which does little more than refine and expand what we already have. There is a need to seek new ways, for bold men who will venture down new paths, for engineers who will approach this problem with a willingness to experiment with the unorthodox.

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* Assistant Secretary, Department of Health, Education, and Welfare, Washington, D.C.

But effective water pollution control is no longer just a health problem or just an engineering problem. The doctors and the engineers can't give us many of the answers we need; they must be found in the laboratory — in the chemistry laboratory, in the biology laboratory.

This is so because the aims of pollution abatement in today's complex society are at least sixfold:

(1) To provide safe, palatable water of the quality desirable for domestic uses;

(2) to maintain and provide water supplies that are safe for agricultural uses such as stock watering and irrigation;

(3) to maintain water supplies of the desired quality for various industrial processes and uses;

(4) to restore or maintain safe waters that are pleasing in appearance and free from undesirable odors for recreational uses such as swimming, boating, and skiing;

(5) to provide water of the quality needed for survival, growth, reproduction, and the general well-being of fresh-water, estuarial, and marine life;

(6) to provide water suitable for power generation and navigation.

It will be seen that most of these have to do with the providing of conditions suitable for living things, plants and animals, and are thus biological in nature and the concern of biologists. As we delve more deeply into the detection of low levels of water pollution and the effects of various pollutants, we find more and more problems that fall within the general field of aquatic biology, the science of aquatic life.

It is in the field of ecology that the aquatic biologist may make some of his most important contributions. In environmental sanitation an ecological concept and an understanding of the environmental factors are of great importance. In complex problems of this type it is essential to work with nature and devise remedial measures that fit into the natural scheme — to work with natural laws rather than against them. Putting into operation procedures opposed to natural processes is a costly, wasteful, and senseless procedure that is certain to result in failure in the long run. All life and life processes are influenced by the environment, and when it is changed through the addition of wastes or some other means, a whole series of events may be set in motion that may have important consequences unforeseen by those alien to the life sciences. One role of the aquatic biologist is to point out environmental relationships and the importance of a favorable environment for all forms of life including man.

We need general recognition of the fact that the surest way to destroy an animal or a species is to destroy its environment or alter it so that it is more favorable for other organisms. It is not necessary to have a directly lethal agent or actually to kill members of a species in order to eliminate it. Modifications of the environment that render it less favorable for the species in question but more favorable for its competitors, parasites, diseases, and predators can eliminate that species just as surely as a lethal agent, even though elimination will occur more slowly. Frogs cannot be raised in a clover field, trout in an eroded arroyo, or bass in a cesspool. Neither can trout be produced naturally in a stream where dissolved oxygen or temperature levels become lethal, even though such levels occur only one day of the year. The aquatic biologist can be of value through continually stressing the fact that the average of environmental conditions is not a measure of the suitability of an environment and that it is the extremes of environmental conditions that are important and are the true measure.

In addition to providing an environmental concept and supplying ecological information, the aquatic biologist can make other valuable contributions in the fields of water supply, waste treatment, and pollution abatement. In these fields he can serve not only in research but also in field surveys and investigations; technical services; the collection, recording, and evaluation of basic data; and in abatement investigations and enforcement actions.

The mention of enforcement actions brings me around to my fellow lawyers. If I seem disposed to blame our doctor friends and our engineering friends for not having accomplished more than we have in water pollution abatement, how can I exonerate my own profession? In the final analysis enforcement actions are the very heart of any effective water pollution control program. Enforcement actions are basically a legal proceeding, always involving lawyers and sometimes the courts.

As many a polluter and many a public health official has discovered, a clever lawyer can undo in very short order months and years of scientific investigation and research. And I would caution you to always keep this in mind. Your work in the field of water pollution control is not likely to be very effective if what you accomplish remains meaningful only to your fellow aquatic biologist. Unless you can succeed in translating the fruits of your labor into something meaningful and important to enforcement officials — be they doctors, engineers, or lawyers — you are destined to remain frustrated in your efforts to contribute to the solution of America's water pollution problems. Your water quality criteria may indeed be impressive in a scientific journal, but how will they sound to a judge, and how will they stand up under cross-examination in open court? You may be great in the laboratory but completely ineffectual in a courtroom. If this is the case, how really successful will you be in your efforts to help solve our pollution problems?

These may be things they never taught you in graduate or undergraduate school, but these are abilities you must acquire if you are to make the full contribution that will be demanded of aquatic biologists in the water pollution abatement programs of the future.

Finally, in order to be consistent, I suppose I should say a few words about politicians. Like everybody else the politician's record in this area is somewhat spotty. Politicians, too, have not always advanced the cause of water pollution control with speed and consistency. It is a matter of record that elected public officials have been known to put budgetary considerations ahead of pollution abatement on more than a few occasions. Many a politician whose public record of support of effective water pollution control programs is beyond question has, I am sure, had moments of doubt when he found these programs being vigorously enforced in his own back yard. As a former member of Congress, I can say that I fully appreciate the mixed emotions involved when a big polluter also happens to be one of your biggest supporters.

But despite any such moments of doubt the record is clear that in recent years the politicians who make up our Federal Congress have been far ahead of the doctors, the lawyers, the engineers, or the scientists when it comes to water pollution control.

To its credit, the Congress of the United States clearly recognized that water pollution problems are not just local programs and that the Federal government has a responsibility to help the local community meet these problems.

To its credit, the Congress of the United States clearly recognized that water pollution problems are not just health problems, but involve broad challenges that have economic, industrial, recreational, and aesthetic ramifications.

To its credit, the Congress of the United States recognized that the engineers and the scientists not only do not have all the answers to our water pollution control problems, but they need help and encouragement to find new answers. This help and this encouragement are inherent in the research provisions that Congress wrote into our Federal Water Pollution Control Law.

In water pollution control matters, in many respects the record of the politician, at least those politicians who make up our Federal government, is the most impressive of all. In my judgment, the politicians have now given the doctors and the lawyers, the engineers and the scientists the basic tools with which to do the job. And what is that job? To bring to an end the wasteful squandering of this nation's most precious national resource — water. To accomplish this end is the basic purpose of the Federal Water Pollution Control Program.

You as aquatic biologists have an essential role to play in that program for, without the knowledge and information that men of your background and training can alone provide, the program can never be a complete success. In the art of pollution control, many of the refinements that must come about if we are to meet our national needs for water can only be contributed by men of your discipline.

As the man charged with the responsibility for making the Federal Water Pollution Control Program work, I say to you that — within proper context — there is no limit to the role the aquatic biologist can play in this essential national program. Why do I say "within proper context"? Because none of us can ignore the realities of life. And some of the realities of life that all of us must be prepared to live with in connection with the Federal Water Pollution Control Program are such facts as: There is never enough money to do everything you would like to do. Our program and your part in that program are in constant competition with numerous other worthwhile efforts. The decision-making process in the bureaucracy is often frustratingly long. Sometimes these decisions are, from your point of view, made the wrong way.

In essence, what I have to say to you is this. The aquatic biologists have not suddenly inherited the earth. In other words, you as aquatic biologists are not about to dominate water pollution matters in the way doctors or again sanitary engineers may have tended to dominate such programs in the past. Instead, you are now a recognized member of the team. As the coach of that team I would say, in all frankness, whether you prove to be a star or a bench warmer is entirely up to you.

VALUE AND USE OF WATER QUALITY CRITERIA

B. B. Berger, Chairman *

THE VALUE AND USE OF WATER QUALITY CRITERIA IN THE FEDERAL ENFORCEMENT PROGRAM. PANEL DISCUSSION

Murray Stein †

Mr. Chairman, distinguished panel members, and friends, for the purposes of those who came late and for certain other purposes, I am going to use Mr. Berger's invitation letter as a point of departure. Here is what he said: "The presentations should be deliberately provocative to elicit a maximum of response from the floor. In other words we want the public agencies, industry, and conservationists to lay their opinions right 'on the line'." Mr. Berger put quotation marks around "on the line." I guess that was to indicate that a man of his deep, scientific erudition and broad international interest and responsibilities knew the vernacular when he talked, but he wasn't quite sure about the panelists. Well, I think that any one of these panelists knew that I would have done it anyway, and as for the other members I think they would, too. But, speaking for myself, I appreciate the fact that you've legalized it and I have an invitation and I'm not indulging a policy of my own. Here are the three questions that were asked: (1) Are water quality criteria for the protection of aquatic life needed for effective water pollution control operations? (2) How can they best be used? (3) What problems need to be solved before we can use water quality criteria for the protection of aquatic life?

If we examine these questions, we see that the second and third assume that the development of water quality criteria is necessary and desirable. It is quite easy to say that water quality criteria are necessary and desirable, but criteria may be one of those double words. We are not quite sure what it means, but I think when we deal with it we have to differentiate between criteria. Obviously, whether you're a doctor, accountant, engineer, aquatic biologist, chemist, or physicist, when you come up with an opinion or make a judgment, you are using professional criteria. There is, however, another type of criteria that some of us may be talking about: criteria developed by a formal process of meetings, discussions, and chewing the cud over and over again. I don't think we can establish the difference between these two types of criteria by saying that when a professional makes a judgment or when an engineer designs a water treatment plant or a sewage treatment plant he is classifying and using criteria, or when a lawyer makes a judgment in court, he is using professional criteria. This is not the same as a group of people getting together in a formal process trying to get a consensus and coming up with criteria.

I don't know what your predilections or your background are—whether you prefer the preacher or the football coach. We do have to get back to fundamentals, however, before we can really begin to understand the problem or its implications when we begin discussing water quality criteria.

When I went to high school and college I was somewhat of an amateur biologist and I have always thought of biology as essentially a descriptive science. There are some people who might try to get away from that tendency, but I think that historically is where the strength of biology is and that is where the great developments have come forth. Great things came from discussions—whether it was Darwin describing what he saw in the Beagle, or Morgan describing what he saw in fruit flies, or Mendel describing what he saw in an Austrian pea garden. Certain great advances in our age have produced both truth and fads. Sometimes these fads have not truly fit in with biology. The truth of our age in a large measure, someone said, is a statistical truth. It is said that the characteristic of our age is a slide. That may be what one of the characteristic truths is—a statistical truth. If you can count something, if you can put it down, people are very much impressed. In very very many places statistical truth has shown it is a remarkable tool in scientific methods. For example, you can take out by mathematical processes the top half of a planet, point a telescope, and there it will be. As you know, new planets have been discovered that way.

The question whether we can apply this notion of statistical truth and reduce a science such as biology to a series of statistical truths needs some real profound thought. I am not sure it can be done in many cases. In one case, for example, I was called by one of the United States Senators who thought that the biologist looking for statistical truth in research problems was carried a little too far. Research projects seem to catch on to the extent that they present something you can count. The Senator said: "I understand the government has approved a project to investigate the rectal temperature of the hibernating bear." I countered by asking how they managed to arrange for the experiment. I never did find out whether the story was true, but this is the end that we sometimes get pushed to when we're dealing with statistical truths.

The other kind of advance of truths involves a bureaucratic truth or the group opinion or consensus. This has certain advantages in that it fits in with our society as the statistical truth does, but it has a few differences. It may fit in with the democratic way of life because of the consensus, but there is a big difference. The group decision does not make a man president when he gets 51 percent of the votes, as our Democratic party has done, nor does a farm bill fail because of a lack of a dozen votes; neither does it give a minority a reasonable time to express a point of view and go ahead. This isn't what happens in the bureaucratic approach. I am speaking not only of government but of big industry and universities because I think by and large all of us—scientists, aquatic biologists, lawyers, and others—tend more and more to work for these organizations. What we must arrive at is a group consensus whereby everyone will agree. This may be fine and this may lead to the development of criteria or it may lead to a formal document, but the relationship between what comes out of this committee approach and getting everyone to agree and establish a scientific truth, is, I submit, sometimes peripheral.

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Some people in charge of organizations like to go to meetings and reach out to arrive at this consensus of opinion. This is a wonderful operation, but the development of criteria in itself does not clean up any pollution. Criteria take a long time to develop because we are not dealing with a specific situation but are taking in the whole world and dealing with all sorts of hypothetical situations. There are really no real social risks involved because we are waiting for a complete consensus, or almost complete consensus, before we come out with anything positive; no one is going to be irritated. At the present time, as some of you may know, Dr. McCallum is in Europe and I am Acting Chief of the Division, and I find it is very easy for a person who is in charge of an organization to enlist recruits with a scientific background so he can go forth with the full panoply of experts to these meetings. To some people this is attractive. I have been to many of these meetings myself, having spent over 20 years in the Government. I grew up and took my apprenticeship going to these meetings. It is something like that great American comedian Fred Allen used to say: "Pull up a flea chair and sit down." You have opportunity for advancement, which a man with a scientific background does have. He can have the pleasure of associating with the top brass of his organization. He can have an unending production of papers; they may be shop papers or cut doilies, but they will be papers. And he may also receive a lot of recognition by mutual admiration societies, euphemistically called professional organizations, if he wants to engage in that kind of practice. Each committee will be another line to add to his thumbnail biography. Now, this is not to say that life isn't tough within this frame, because within it life is real and life is earnest and careers are made or broken. As a matter of fact a complete transformation takes place when the person engaging in this can't recognize any tempest outside of a teapot. These people spend their lives in mental gymnastics. I do not say they are not creating a useful social school, because this has to be done. We will always find people who will want to do this, and it is good in our society. What we have to consider is, however, the other men, the men who want to achieve in a scientific manner.

There are several problems we know we have in biology. This science is different from the other sciences and it is not as easy perhaps to develop criteria in this field as in other fields. For instance, biology is a life science. The chemist and physicist can reasonably know what happens here or in another world or in another galaxy or predicament. The biologists are not quite sure whether life is not a unique panacea. Whatever your feelings about the deeper mysteries of life—whether spiritual or mechanical—we do know that a live force that has a will of its own and moves on its own in an unpredictable manner creates complexities that are different in a measurable degree than those of some of the other sciences. We do know that you in the biological sciences, in aquatic biology in particular, are dealing with a complex ecology; you know how complex it is, and the introduction of outside elements creates something that is ever changing. We know, for example, that use of DDT affects the fly and that the change in a single week in an interrelated living process can make tremendous changes in a complete environment, often in unpredictable ways. We are seeing in the newspapers what a lot of us knew in the past about the unexpected side effects caused by the introduction of new drugs or chemicals into an environment.

These are the things we are aware of in the biological sciences. Sometimes it isn't easy to transform them into a statistical formulation. The mission of the aquatic biologist in the water pollution control field is to leave us with water a little bit cleaner, or to leave us with a little more knowledge. At the present time his challenge in dealing with a life science and a descriptive science is to describe the effects of pollution and the causal relationship between the elements that create these effects, on a case-by-case basis, with the objective of abating or preventing pollution. The compiling of water quality criteria will follow. Aquatic biologists have a twofold mission: First, they are protectors—the first line protectors—of a vital natural resource, the biota of our streams; second, they can use the aquatic life as indicator organisms in order to specify fairly detailed pollution control measures and damages. Throughout the country one of the big problems we have now is a proliferation of municipal and industrial waste treatment plants that may be adequately designed, but do not work effectively 365 days a year. If our electric utilities do not have enough capacity and fall apart on a winter day, we hear about it soon enough; however, when a water pollution control plant fails or doesn't work for a few days, we do not always know about it. How to determine whether a plant of this nature is operating effectively all the time is a challenge for the aquatic biologist. I think he has enough technical knowhow and knowledge to make a judgment about whether a particular aquatic environment is being injured below a point of discharge, or what the situation would be without that point of discharge.

I am not against the development of criteria. What I am suggesting, though, is that we guard against allowing the development of criteria to be the main force of our professional life. Too often an organization's time, budget, and staff are completely occupied in developing water quality criteria while they neglect the job they are created for, which is to protect the quality of our waters. One example, a fish kill, is rather dramatic. I am not even dealing with the more discreet effects on fish. Time after time I have heard aquatic biologists complain that they were not notified in time, that they could not get to the fish kill, and that they did not know what caused it. But I have not heard of a motion at any meeting to set up a technique or methodology or something that would bring biologists right to the spot after the fish kill on a case-by-case basis. We could get information. The point I am making is that first things come first. There is no doubt that criteria are very important, but let's not forget that we have to get the basic building blocks in shape before we move them forward. I believe, too, that the people who have participated with us in an active program to clean up pollution—that is, those who get out on the firing line in the field on a case-by-case basis, get into the stream, see what is happening, make their report, stand up before God and country, if necessary, certainly before a hearing board or in the courts—have found it stimulating. They have found great satisfaction in it; they have found that by getting out into the field and being closely questioned they have come up with new insights for research and presentation. As one person in the room told me while preparing for a case: "You know, it is quite a different thing preparing my presentation for a case where I'm going to be cross-examined and one going before a scientific society." I think you should try possibly to give yourself, if you have the courage, the pleasure of going through with them and seeing what that does to you and how it sharpens up your thinking. I think that participation in an active program leads to new concepts and this inevitably will develop criteria with force and meaning. Too often have I seen criteria developed among opposing sides and some high administrator whose major art is bringing together differences or splits. This may be the way to develop a good social instinct, but whether this is the way to arrive at scientific truth, I leave to you.

I am suggesting to the biologist and to the aquatic biologist in particular that we need your help in cleaning up this nation's polluted waters. In every enforcement case we have had under the Federal program, effect on fish life was a problem for which we needed the help of a biologist. I am suggesting that in the process of cleaning up pollution and developing comprehensive programs we can more readily arrive at scientific truth than we ever can in a meeting with representatives from scientific societies, and governmental organizations, and industries, no matter how exalted they may be.

THE VALUE AND USE OF WATER QUALITY CRITERIA TO PROTECT AQUATIC LIFE IN STATE PROGRAMS

Milton P. Adams *

My connection with state water pollution control efforts, as most of you know, is based for the most part in Michigan. That period of service extended from October 1, 1930, to July 1, 1962, on which date I retired from state service. Notwithstanding that change of status, both Clarence Tarzwell and my successor at Lansing, Larry Oeming, felt I should make this presentation. So here I am, accompanied by one of your active co-workers—an exceedingly industrious biologist and highly prized member of our staff—Carlos Fetterolf.

State laws, parameters, tests, standards, and restrictions changed considerably during my period of service. Many are of the opinion that despite progress made to bring present and past waste and pollution under control, a losing battle has been waged by the states. This accounts for recent strengthening of Federal pollution legislation (Public Law 87-88) and for action taken under this authority.

It appears from a review of the transactions of your 1956 and 1959 seminars that the subject before this panel is not a new one. I really think you biologists already know the answers. You're waiting to see if we make a mistake—a federal man, a state administrator, a spokesman for industry, and one for wildlife. Several interests seem to be omitted, however, from the ranks of the panelists and in the restrictive title of this program. Water quality criteria is a subject for discussion here only as it relates to the protection of aquatic life. Perhaps it is to be assumed, or can be shown later, that if aquatic life is protected then all other uses such as agriculture, recreation, public health, and the like, will be cared for as well. For many years, I tangled with an industrial lobbyist at Lansing who always asked me "What's more important—payrolls or fish?" My answer was always the same: "We have to have both."

Chairman Berger requested each panelist in his presentation to consider three questions. In answer to the first one, "Are water quality criteria for the protection of aquatic life needed for effective water pollution control operation," my answer would be either "Yes and no" or "Yes, with certain reservations." These I would now discuss.

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As previously stated, I dislike to see the criteria under discussion limited to the protection of aquatic life only. This throws the state administrator off balance at the outset. If he needs criteris for the protection of aquatic life, he needs them equally for the protection of other lawful uses of the waters of his state. Across this broad land of ours differing views are held by administrators about the value of standards, objectives, criteria, parameters, and the like. Some of these differences arise as the result of contrasting laws or public policy with respect to wastes and pollution within the 50 jurisdictions; others are due to the scarcity or inadequacy of background information.

None of the states have had the resources for conducting surveys, background studies, planning, and future projections that are available, for instance, to the Surgeon General in the Great Lakes-Illinois River Basin project or to the Secretary of Health, Education, and Welfare as he lays a future enforcement foundation for correcting such ills and shortcomings as exist in the protection of the Detroit River and Lake Erie.

Most state adminstrators necessarily regard criteria, or objectives, or standards as means of facilitating compliance with their state laws. In Michigan, for instance, our law is almost silent on advance planning but forthright in declaring what is unlawful. Discharged substances injurious to the public health, and the conduct of any industrial enterprise or other lawful occupation that results in discharges that destroy or prevent the growth or propagation of any fish, migratory bird life, wild animal, or aquatic life are all specifically mentioned—even the value of lawfully taken fish or game, impaired as the consequence of pollution, are included. You will immediately recognize an opportunity for development of such criteria and parameters as MPN's, DO's, BOD's, pH's, phenols, cresols, oils and greases, cyanides, chromium, and the like.

On the assumption that all surface water supplies for public use must be filtered and chlorinated, we may also encounter 1,000 MPN or less for acceptable bathing beaches, and 5,000 MPN or more for sources of public water supply.

It is significant, I think, that in the enactment of state laws with their varied prohibitions or lack of them, such things as water quality criteria are avoided. Maybe this is because few legislators understand what a zero, 2, or 5 ppm DO in a natural water would mean, or a million coliform index. Looking back over my 32 years in Lansing, during which I served six State Health Commissioners, I have been aware of widely varying concepts of what water qualities would prove injurious to the public health! And we still lack a solid definition.

While we, as administrators, and our supporting cadre must have water quality criteria as target objectives in our work, however they may be expressed, we would be less than realistic if we failed to recognize their limitations as well as advantages when it comes to solving a given problem.

In the early thirties, the degradation suffered by the prize coon dog of Jackson County after swimming across Grand River below Jackson, as related by his master before the visiting Circuit Judge, won a court decision for a lower riparian plaintiff. My explanation of charts made after an exhaustive pollution survey by my staff depicting the rise and fall of DO and BOD elicited from Judge Parker only the words "DO, BOD, B coli—Bah!" You will understand then why in the midst of their technical survey work I have dropped the suggestion to our boys "be on the lookout for a convincing lay witness—remember the coon dog."

The Commission is being given a very hard time right now in an extended paper mill hearing. This grew out of staff and Commission adoption of a minimum DO criterion for the central section of the Kalamazoo River. It makes no difference that the other eight managements in the valley have stipulated and agreed to reduce their present waste contributions to equal 39 percent of the 1950 contribution of each. Pro rata method accepted all around would provide 2.5 ppm DO when the river is at its 10-year frequency of low flow. The staff and Commission have had to take account of the particularly severe condition of stream use imposed by the concentration of pulp and paper mill production. It cannot "give ground" on the target objective of the remaining mill without reopening the case of all others. So it's clear that official and extended proceedings under our law grounded on the application of water quality criteria have failed, so far at least, to bring results in the central Kalamazoo River section.

Finally, a third example might be mentioned where objectives set after careful studies have failed to correct boundary water pollution between the United States and Canada. I hasten to add, however, this is not the fault of the criteria set up by the International Joint Commission in 1950 and adopted by both Canada and the United States in 1951 but an apparent breakdown in communication between Federal and state government on our side of the boundary and between national and provincial government on the Canadian side.

So here it must be concluded in three cases that water quality criteria set up as compliance aids have failed to produce results for one reason or another.

In answer to the second query of our Chairman: "How can they best be used?", I conclude thay they can be used (1) as administrative, rather than enforcement aids and (2) as aids to the implementing of such provisions of law as are found in Section 2 (a) of the Federal Water Pollution Control Act. Here the interrelationship among Federal, interstate, state, municipal, and industrial agencies to obtain certain results through comprehensive programs for water pollution control clearly requires soundly conceived criteria that are susceptible of fulfillment.

Finally, in answer to query three: "What difficulties presently impede the development and use of water quality criteria for protecting aquatic life?", I would answer "lack of knowledge." Basic and applied research for the most part would seem to be the answer here. This research should supply the enforcing agency with solid answers to back up their position when restricting waste loadings. Industries and municipalities, as well as the enforcing agency, need to know what the effect will be when and if limiting criteria are exceeded.

Based on our experience, our conclusion is, therefore, that water quality criteria to protect aquatic life are useful tools for a state agency for planning and administrative purposes. They may or may not prove effective as an aid to enforcement. Finally, much is still to be learned through research to substantiate present assumptions and lay the groundwork for future declarations of value.

THE VALUE AND USE OF WATER QUALITY CRITERIA TO PROTECT AQUATIC LIFE: A RATIONAL INDUSTRIAL VIEWPOINT

Roy F. Weston *

The speaker has been requested to give industry's viewpoint on this complex subject. Surely, it is recognized by all that no individual can hope to present the many diversified and sometimes conflicting opinions of the individual representatives of industry. There are no members of a group that typify rugged individuals more than those representing American industry. Nevertheless, there are factors common to most industrial operations that make it possible to rationalize an industrial viewpoint. I have, therefore, chosen the sub-title "A Rational Industrial Viewpoint." The discussion below is my interpretation of a rational industrial viewpoint relative to the development, establishment, and enforcement of water quality criteria.

Water quality criteria for aquatic life cannot be considered alone. They must be considered as a specific part of a broad spectrum of criteria pertaining to numerous different water uses. Their mode of application must conform with a general and sound philosophy of pollution control. Their enforcement must be reasonable, equitable, forceful, consistent, and persistent.

Commercial and sports fishing and hunting and the recreational use of waters require proper protection of aquatic life. These are reasonable and legitimate uses deserving of protection. Therefore, water quality criteria must be established to protect these uses. Such criteria must be considered essential to an effective water pollution control program.

Establishment of such criteria is required if industry is to know specifically what is expected of it.

Of course, it must be remembered that aquatic life requirements are but a few of the many use demands on our natural waters. They must be considered in their proper perspective.

There is competition for water. It should be used in a manner that protects the rights of legitimate users and is of best use to the greatest number. In other words, the use of natural waters should be in the public's best interests.

Most industrialists will subscribe to the above statements. Philosophical concept, however, confuses the issue from now on. There are those who contend that economics must control water use. They belittle the value of a "few fish." They expound with confidence that jobs are more important than fish. They state that we must choose between fish or people, between fishing and working. These people are sincere in their beliefs.

Such views are not necessarily industrial. They come from many different walks of life.

The rational views of the industrialist present a special case.

The average industrial manager is interested in pollution control for three interrelated reasons:

1. To maintain prosperous business levels through good public relations;
2. to prevent losses from forced plant shutdowns, costly lawsuits, or claims for damages;
3. to reduce costs caused by lowered water quality.

These are practical factors unassociated with altruism.

The corporate entity is created to serve society and to reward its owners for such service. It cannot exist if it does not make a profit.

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The primary purpose of the industrial manager is to assure a profit for his employer. He is trained to demand due justification for every expense. He invests capital only on reasonable assurance of a high rate of return.

Water pollution control normally requires investment without return. In fact, it generally increases operating costs in addition to increasing fixed costs. Thereby, water pollution control reduces profits. In fact, water pollution abatement at an existing plant may so alter its economy as to create serious economic problems. Therefore, the industrial manager is obliged to abate pollution at minimum cost and to delay such costs as long as possible.

It should be obvious that the nature of his training, experience, and general philosophy influences the industrialist's views and policies concerning water pollution control. The most reactionary industrialist will have the "job versus fish" philosophy and will act accordingly.

The most public-spirited industrialist cannot escape his inherent philosophies, training, experience, and responsibilities. His policies must, therefore, be controlled by the hard facts of economic realism.

This does not mean that the industrialist has no social conscience. He does have a social conscience. Twenty-three years ago, when I was employed by an industrial concern to work on their water pollution abatement problems, I was advised that the executive head of that company stated the following philosophy: "The cost of a company's product to the public includes the price paid for the product plus such damages or other costs that may accrue to the public as the result of the company's operations, e.g., costs of water pollution." In view of the times, this was a most enlightened viewpoint. Competitive economics being what they are, however, this viewpoint cannot be carried to extremes. Consequently, industry is forced to use the philosophy of "enlightened self-interest."

Because the costs of pollution abatement affect the profitability of the industrialist's operations, it is reasonable that he should adopt the philosophy of providing no more reduction in pollution than is necessary to accomplish an immediate and essential objective. He will also reason that a balance should be made between costs to industry and benefits derived. Most enlightened industrialists, I believe, will agree that simple evaluations such as the cost of pollution abatement per pound of potential fish production from a given stretch of stream cannot be used to justify pollution abatement. On the other hand, many industrialists may reasonably resist demands to treat their wastes to the extent necessary to make a short stretch of a small stream acceptable for normal aquatic life. This is particularly true if there is no other compelling reason for such a high degree of treatment.

This means, in my opinion, that the rational industrial viewpoint subscribes to the philosophy that pollution should be evaluated on the basis of socio-economic effects as well as effects on water quality per se. In any case, the viewpoint would require "reasonable use" of available resources. For instance, a waste water may be lethal to aquatic life without dilution but harmless in the stream after dilution. The use of stream dilution in such a case should be a legitimate and reasonable water use.

This philosophy is sound economically. Obviously, it raises many complex technical questions. Some of these questions are:
How shall acute toxicity be evaluated? How shall chronic toxicity be evaluated? What test procedures shall be used to establish a biologically safe concentration? What organisms shall be used as test animals and plants? What factor of safety shall be used in applying test results? Who shall develop and establish the necessary standards for testing and applying test results? How shall variation in streamflow and waste water quality be considered in establishing allowable safe discharges? How shall allowable discharges be allocated among industries, and among companies of an industry? These are but a few of the many practical questions that must be answered.

Industry, I am sure, subscribes to the need for water quality criteria to protect aquatic life. It will expect that such criteria be reasonable and as specific as possible. It will expect to be required to meet only those criteria that the regulatory agency is prepared to monitor and to enforce.

Industry realizes that in many cases regulatory action must be taken prior to the solution of all technical aspects of a control problem. Nevertheless, it does expect professional responsibility in the development of water quality criteria. Many industrialists feel better if they are a party to the development and establishment of such criteria. The cooperative industry-regulatory agency development of the temperature criteria in the State of Pennsylvania is frequently used as an example of how such things should be done.

It should be expected that criteria cooperatively developed will be technically sound and will clearly define conditions of use. Procedures should be available and specified for estimating biologically safe concentrations of chemicals or wastes. Moreover, procedures to be used for determining compliance with the criteria should be specifically outlined. Such procedures and information are necessary for the design of treatment or control facilities, or both, and for checking compliance.

The establishment of sound criteria and supporting test procedures is highly complex, particulary if both chronic and acute toxicities must be covered. Just as complex is the application of such criteria. Two basic areas of responsibility are involved.

1. The establishment of biologically safe concentrations for the specific chemicals or combinations thereof in an industrial wastewater discharge.
2. The establishment of the allowable discharges of specific chemicals or combinations thereof from individual plants.

It is believed that the first area is the responsibility of the industry involved. The burden of proof for establishing the acceptability of an industrial waste discharge rests upon industry. This is based on the philosophy that the discharge of waste is a privilege rather than a right. This argument may be countered with the theory that a polluter is innocent of causing harm until proven guilty.

The establishment of allowable discharges is a regulatory agency's responsibility. Since the industrial community on a stream is dynamic, the regulatory agency is faced with a complex problem in equity. For instance, at this particular time, a stream can be adequately protected, in the case of minimum 5-year flow, if each plant removes 50 percent of the toxic materials from its wastes. Adequate protection could be assumed if the biologically safe concentration of a toxic chemical existed for normal industry waste water flow and concentration. Now, let us assume that Plant A and Plant B make the same product and discharge the same type of waste. Plant A, however, discharges twice as much toxic chemical per unit of production as Plant B, because of poor in-plant control facilities and procedures. This factor should, therefore, be considered in determining the degree of treatment required by Plant A as compared with Plant B. This problem can be simply and equitably handled by relating the allowable discharge of toxic chemicals to production.

Now, if Plant C discharges the same toxic chemical as Plants A and B (for the sake of simplicity), but makes a different product using different processes, it is apparent that the regulatory agency is faced with a difficult problem of allocation of allowable chemical discharge. In those cases in which simple direct comparisons cannot be made, other criterion for comparison may be provided, such as net worth to the community, by which payroll, taxes paid, or some other basis is used for comparative purposes. In time, equitable criteria for determining such discharges must be developed.

There are those who will argue that the theory of "reasonable use" of a stream's pollution-carrying capacity is unreasonably complex and impractical from an administrative viewpoint. They would prefer to use the expedient of requiring all industry to provide the highest practicable degree of treatment available so as to discharge the minimum practicable quantity of pollution. This concept is subject to arbitrary rather than rational decision making. It can be uneconomical and mysterious, confusing and frustrating to those subject to its enforcement. The philosophy of enforcement and control should be easily rationalized technically, economically, and equitably.

In a general sense, all regulatory agencies recognize that relatively small quantities of pollution can be permitted in a relatively large stream. Therefore, our large rivers receive effluents from primary treatment plants in some cases and in many cases receive polluting materials that have been unaltered prior to discharge. In other cases, a higher rate of waste discharge is allowable at flood stage than at low flow. Moreover, municipalities and industries that discharge into the sea frequently are required to provide primary treatment only. These and many other illustrations indicate that historically and in current practice those who administer stream protection laws recognize the concept of "reasonable use."

In many river basins, regulatory agencies are currently faced with the problem of equitable allocation of allowable pollution load because the pollution-carrying capacity of the stream is relatively small compared with the discharge of polluting materials. Unfortunately, they have little precedent for sound decision making. This situation will occur and reoccur in the future.

In consideration of established practices and the problems of the future, it is quite obvious that the development and use of water quality criteria to protect aquatic life present highly complex technical problems and equally complex administrative problems. The establishment of sound criteria will require the team approach and the best talent available in the biological, chemical, hydrologic, engineering, and administrative professions. The criteria developed must be reasonable, equitable, measurable, enforceable, and enforced. Considerable research effort is necessary to assure the development and sound application of such criteria.

It is believed that the industrialist recognizes that water quality criteria are essential to a sound water pollution control program. He is aware that such criteria can be of significant assistance to him in determining his pollution abatement problem, in defining his treatment or control requirements, in determining his capital budget needs, and in designing and operating his treatment or control facilities.

He trusts that the development of such criteria will recognize and define the concept of "reasonable use." He understands and respects those criteria developed by industry-regulatory agency cooperative effort. He acknowledges the need for regulatory action to abate pollution prior to resolution of the many technical and administrative ramifications inherent in the development, establishment, and enforcement of sound criteria.

He recognizes that the development and administration of water quality criteria pose a great challenge to the water pollution control profession. He must urge that profound thought, extensive research, and responsible action be the basis for the development, establishment, and enforcement of water quality criteria.

THE VALUE AND USE OF WATER QUALITY CRITERIA IN CONSERVATION PROGRAMS

Thomas L. Kimball *

Today there are 66 million-plus outdoor hobbyists. In pursuing the pleasures of outdoor recreation, the activity of most of these teeming millions is oriented on or adjacent to water.

By the year 2010 it is estimated by the National Part Service that the demand for outdoor recreation will exceed present activity by 40 times.

The United States Geological Survey reported in a news release from the Department of Interior on May 7, 1962, that the use of water in the United States increased 12 percent between 1955 and 1960. The report went on to state that 270,000 million gallons of water per day (mgd) was withdrawn for a multiplicity of uses, but only 61,000 mgd was consumed. Approximately 77 percent of the total water withdrawn for use was returned to the source, much of it in a deteriorated or polluted condition.

The Senate Select Committee on National Water Resources reported the following on the amounts of water needed for fish and wildlife purposes.

It is estimated that by 1980, 66.7 mgd would be needed for consumptive use to maintain important wetland habitats for waterfowl and other species of wildlife; by 2000, 89.9 mgd will be needed for the same purpose. In 1955, the maintenance of sport fish habitats required a flow of 78 mgd. By 1980, the need to enhance stream fisheries to meet the demand will require 171 mgd, and by 2000, 241.4 mgd.

In 1954, it took an estimated 512.4 mgd for waste dilution to maintain dissolved oxygen at an average of 4 milligrams per liter. This estimate was based on a 70 percent treatment for municipal sewage and 50 percent treatment for industrial wastes, both higher than actually prevailed. If it is assumed that pollution abatement will proceed as scheduled, it is estimated that 332.2 mgd will be needed for waste dilution in 1980 and 446.5 mgd by 2000.

These facts are mentioned together for three specific reasons. The first is to draw attention to the popularity of water-oriented outdoor activity; practically all of our adult population participates in some form of outdoor recreation. The second is to show the ever-increasing demands on our water supply for a wide variety of uses, and the third is to point out that waste dilution in 1954 was the largest single use of water. Perhaps a specific and typical example of water wasted for pollution dilution would be enlightening.

The United States Corps of Army Engineers recently presented a plan for the development of water in the Potomac Basin. The program called for the construction of 16 high dams within the Basin at a cost of several hundred million dollars. The primary objective of the plan was to establish a continuous delivery of 3 billion gallons of water per day (gpd) for domestic, industrial, and recreational use and for water quality control for the Washington, D. C., area. Fully two-thirds of this flow, or approximately 2 billion gpd, was needed for pollution dilution. This information immediately raised the question in the minds of many public-spirited citizens whether or not funds allocated for pollution dilution might be much more wisely spent in pollution abatement; this would permit re-use of the amount of water needed for waste dilution, while saving many fertile and productive river bottom lands and many important areas of historic and scenic value from inundation.

These statistics illustrate that water demands are increasing in geometric proportions and, if all water needs in the public interest are to be met, we cannot long continue to render unfit for re-use any portion of our available supply. Assuming this to be a worthy objective, let us explore the possibility and mechanics of accomplishment.

Water experts have stated on occasion that "the dependable supply of water is not a fixed amount but can be increased by surface storage, and the reduction of evaporation and transpiration losses or other forms of waste." Conservationists are most anxious to maintain our watersheds in a condition that will insure soil stability and a sustained maximum yield of quality water. This goal can be attained only through wise use and management of the timber, forage, and mineral resources. Pollution abatement, however, offers the most difficult challenge and a real opportunity to provide the greatest single contribution toward an increased, usable water supply.

The Congress recently amended and strengthened the Federal Water Pollution Control Act. Additional funds were made available as grants-in-aid to states and municipalities in an effort to encourage adequate sewage treatment. Federal enforcement authority was increased and enlarged to cover both interstate and, where local and state authorities had demonstrated an inability to cope with pollution problems, intrastate waters. No law is effective without adequate enforcement. No enforcement of water law can be adequate without a definite set of standards established by competent research.

The United States Public Health Service has done a creditable job in establishing standards of water quality for the protection of human health. From the conservationist's viewpoint, however, these standards seem based on a premise that permits accumulation of waste materials in water up to the point where such contamination adversely affects human health. Very little information is available on the concentrations of various pollutants that are harmful to fish, wildlife, and recreational potential. This position, which permits pollution to the point of direct harm, can be readily understood since removing all deleterious substances from water to create a dependable supply for a wide variety of uses is expensive and depends on the ability and willingness of the user to pay the costs involved. It is my contention, however, that the time has now arrived in the over-all population growth and economic development of our nation when we can no longer afford to permit a water user to withdraw any quantity of water from our lakes and streams without requiring him to return it to the source in such quality as to permit its re-use by all other legitimate users.

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* Executive Director National Wildlife Federation, Washington, D.C.

As a conservationist and one interested primarily in the welfare of our nation's wildlife, let me now discuss the value and use of water quality criteria in conservation programs. As the former Director of Game and Fish in Arizona and Colorado, I had the opportunity to struggle with the administration and enforcement of state pollution laws affecting wildlife. Both states have what is considered by most observers to be an effectual and efficient anti-pollution law. Relative to the wildlife resource, the Colorado state law contained the provision that "no deleterious substance adversely affecting fish shall be permitted to enter the waters of the State."

After a few court cases it was soon apparent that the burden of proving substances or effluents deleterious to fish life was the difficult task of the prosecution. The mere fact that thousands of fish were found dead was not sufficient evidence that certain pollutants were the cause. Fish population studies had to be conducted above and below suspected sources of contamination. Proof of fish losses was further complicated by the fact that most fish killed by pollution usually sink to the bottom of the stream or lake.

Laboratory work simulating polluted water conditions had to be initiated. Intense studies of the entire aquatic biota by highly educated and professionally trained personnel who could qualify as experts on the witness stand became a necessity.

An understanding of the interrelationships of aquatic vegetation, associated organisms, and insects that meet the dietary requirements of a wide variety of fishes is essential in the development of water quality standards designed to protect the fisheries resource. In fact without such information successful prosecution of pollution cases is difficult if not impossible.

The number of technically trained people capable of developing water quality standards for the protection of aquatic organisms is woefully inadequate at both the state and federal level. What is needed is a sufficient research staff with adequate laboratory facilities to develop water quality criteria for all recreational uses of water as well as for industrial and public health purposes. Standards so established and backed by competent research work can exercise a telling influence for good on pollution before it happens and exercise a potent and authoritative effect in the courts on those who insist on flouting the law. All water users have a right to know in advance what effect possible pollutants will have on the relative quality of water. If this type of information can be provided to new industries, positive preventative pollution abatement can be initiated.

Coordination of effort and the elimination of duplication among sanitary engineers, doctors, and aquatic biologists at the local, state, and national level are desirable. We need to develop water quality standards for all legitimate uses of water, including all types of outdoor recreation. We should not rest until we have the trained staff, the laboratory facilities, and ample funds to do the job.

Even when water quality standards have been established, the fight will not yet be won. There will be reluctant district attorneys, attorney generals, governors, members of congress, and officers within the executive branch of government who will be unwilling for personal or political reasons to prosecute to the full extent of the law for the maintenance of clean water. There will be individuals, partnerships, corporations, municipalities, industrialists, and portions of the great American public who will be unwilling to pay the price for research to produce standards and the necessary development and construction to obtain water quality. We cannot, in my humble opinion, procrastinate any further. We must have enforced water quality standards to meet the ever increasing demands of the many and varied uses of this important renewable resource. We must eliminate the great American tragedy of utilizing for waste dilution the greater percentage of water withdrawals from our limited supply. Anything less will inhibit the economic and cultural growth of our nation and place us among the historic civilizations who wasted and squandered their national inheritance.

THE VALUE AND USE OF WATER QUALITY CRITERIA

DISCUSSION

Mr. Beak opened the discussion by expressing the opinion that water quality criteria could be developed without interfering with the solution of everyday problems. He felt that research and day-to-day operations should not be in competition. Research should be carried on for the setting up of criteria that can be used in the field. He strongly asserted that more knowledge is needed if we are to improve our field methods, which are much in need of improvement.

Dr. Ronald Allanson, National Institute for Water Research, Republic of South Africa.

I am interested in what has been said by the panel, particularly by Mr. Kimball, because it is exactly what we in South Africa have been endeavoring to put into practice. I think it might be of interest to the members if I outline the way in which we work. There is a tendency to place upon industry the responsibility for the purification of their wastes. The purification of wastes is expensive, but as I said to one of our gold producers at a meeting of the Committee, it may be necessary. This company receives water from a big private water purification system, and they pay a shilling per thousand gallons. At that cost they can economically produce gold, but with an increase in pollution and the consequent purification that would be necessary, they would have to pay more than 2 shillings per thousand gallons for their water. Under these conditions, the price of gold and the cost of mining it would be uneconomical. Industry in the Republic of South Africa is nowhere near the size of that in your country and nowhere near as complex, but in miniature we have a very similar problem. We have national and provincial governments similar to those in Canada. There is a Council of Water Affairs, which is responsible for all water uses in the country — recreational, industrial, and municipal. This body is advised by the Bureau of Standards, which standardizes all sorts of things, even the quality of Coca Cola. They have standardized the quality of effluent that may be discharged into rivers. I'm extremely interested in Mr. Kimball's remarks that what Americans should do is think and work actively so as to return water eventually to its source in a condition suitable for another use. The Water Act of 1955 for the Republic of South Africa indicates very clearly that water used by any industrial activity must be returned, if possible, to the original watercourse from which it was derived, or to another watercourse. The Act also includes sewage works as part of an industrial undertaking. The standards promulgated in South Africa by the Council of Water Affairs through the South African Bureau of Standards have rested very clearly upon intensive research in the field of river biology, river chemistry, and river physics, which has been carried out by the Research Institute that I represent here at this meeting. These standards are from your point of view unrealistic. For example, the standards specify that the effluent must not contain more than 100 fecal E. coli per liter, one hundred percent of the time; that it must not contain more than 10 ppm of ammonia nitrogen, or have a BOD of more than 10 ppm. Industry is of course appalled at this standard, but the way we work with industry is to present to them that this standard is a basis for assessing violations, but that exemptions can be made and the Act acknowledges that exemptions can occur. We urge industry, whether it be a local product manufacturer, a sewage works, or a big national industry like the steel industry, to apply to the Department of Water Affairs for exemption from one or all of these standards. Exemptions are allowed only after consultation around the table by the industry, the research organizations concerned, the Institute, and the Bureau of Standards, together with the Department of Water Affairs. We ask the industry what it is physically able to do at the moment and based on discussion we recommend to the Ministry of Water Affairs that an exemption be made specific for the particular item that varies from the general standards. We lay down one condition, however, and that is that this particular industry, whether it be a large national government body or a small private place, must attempt in the following 5 years, or however many years we decide, to improve the quality of its effluent so that it comes nearer to the general standards. This might sound complicated and rather idealistic, but I can assure you that it works. I remember one case of a steel corporation in South Africa that was appalled when we laid down certain requirements for their responsibility to the national group; I was most gratified to sit on the Committee a few weeks ago and realize that the report we had before us represented a very real improvement in their whole water effluent purification. But, as I say, this can be done in South Africa because we have this organization. It is in essence what Mr. Kimball is asking for and what we in South Africa are trying to do. I must say, and again support Mr. Kimball, that water quality standards must rest upon fundamental and applied research. If the biologist and the chemist and the physicist are called in at every possible level, this coordination will work.

Mr. Stein

There was one remark I omitted from my talk and I am, therefore, very happy you brought this up, Dr. Allanson, because you and Mr. Weston have made some remarks that put this problem in focus. Mr. Weston was speaking about the establishment of safe concentrations. You don't get BOD's when you talk about the establishment of safe concentrations or allowable discharges. I might point out from a logical point of view that this is not the only way to establish criteria; that is, to dump into the stream as much as you can possibly get away with. The other technique is that exemplified by the Food and Drug Administration where, if an element is considered deleterious or poisonous, even a trace of it cannot be introduced into a food. I think the South African research possibly tends more toward the Food and Drug approach. Mr. Weston's experience tends more to what we assume here when we think of criteria. In your deliberations, you should not forget that. Another approach in establishing criteria is to put them at a minimum, and not necessarily assume that the only type of criteria are those, so prevalent in this country, that allow everything possible to be put into a stream.

Mr. Kimball

You might say, Dr. Allanson, that that particular experience is unique to your country. We have similar problems here in the United States about the use of chemicals for insecticides, pesticides, and other purposes. This matter, again, is quite a challenge to the team of research scientists. To my way of thinking there are other chemicals that possibly could control aquatic vegetation in certain ways and not be deleterious to fish life or to the biota in a stream. The problem here in America is that we just haven't had sufficient money allocated to the research teams to provide this type of information. I am quite sure that our team of scientists is up to any challenge put to them. Certainly if we can put men in space and if interplanetary travel is something that can be looked forward to in the near future, there isn't anything beyond accomplishment as far as improved technology in any of these sciences is concerned, given the necessary funds and time.

So if the problem is one of agriculture vs. fisheries or recreational developments, there are other ways and means of desirable control that still protect the fisheries values of the stream. To me at least this is the answer. We do not have to make a choice between fisheries and navigation, for example, by elimination of a plant at the expense of the fisheries. In my opinion, if given the proper time and money, we can have both with improved technology.

Dr. Tarzwell

I believe we need to define some of our terms, for misunderstanding appears to exist. It is quite apparent that there is no general agreement as to what is meant by pollution, toxicity, and water quality criteria. The term "pollution" is often very loosely used and appears to have different meanings to different groups. To me, water pollution is the addition of any material or any change in character or quality of a water that interferes with, lessens, or destroys a desired use. Pollution is, therefore, tied to use; if there is no interference with a use, there is no pollution. When we say a material is a pollutant or that a stream is polluted, we should be indicating that a use of the water has been adversely affected.

"Toxicity" is a quantitative term. Many materials become toxic if they are present in high concentrations. Even common table salt, an everyday necessity, is toxic if taken in large amounts. Many of you recall the recent poisoning of babies in a hospital when salt was used instead of sugar. Several materials that are potential toxicants are required in small quantities by many living things. Certain trace elements are essential for life. Phosphorus, nitrates, copper, zinc, sulphur, molybdenum, potassium, manganese, boron, and chromium are all necessary, but when they exceed certain levels they become toxic. An outstanding example is selenium; it is extremely toxic, but it has been found to be one of the most important trace elements essential for life. In short, many materials must be present in the water in small quantities if we are to have aquatic life. These and many other materials become toxic only when they exceed certain concentrations. In view of these facts, how can we say no toxicant shall be added to a stream? I believe we must say that these materials must not exceed a certain concentration in the stream. Why pay the cost of complete removal from waste waters if these materials occur naturally in the water and are needed? Removal is a costly process, especially the removal of that last small fraction of a percent. Why pay the extra cost for complete removal of that small amount that is not harmful?

Now, as to the meaning of "water quality criteria." The purpose of water quality criteria for aquatic life is to maintain or restore those environmental conditions that are essential for survival, growth, reproduction, general well being, and production of a crop of the desired aquatic organisms. Since the purpose of these water quality criteria is to maintain or restore a favorable environment, the criteria must be based on a thorough knowledge of the environmental requirements of those organisms we seek to protect. Our knowledge of these requirements is limited. We need to know much more. This means a research program. Detailed toxicological information cannot be gained by field observations alone or by a case-by-case study of fish kills. We have been doing that for 50 years and we haven't come up with the answers.

With our present knowledge, it wouldn't make much difference whether or not a biologist were present during a fish kill due to a toxicant. Generally, he couldn't determine the cause in the field. At present, neither biologists nor other groups can tell much about the cause of fish kills. We can tell if the temperature is too high or the oxygen too low, if they are really high or low. We have developed a method of detecting cyanide, but it was developed by laboratory research. Recently, in our Newtown laboratory, we developed a method of determining kills due to heavy metals. Water quality criteria for aquatic life can be developed only through research.

I have never suggested that environmental requirements be arrived at by a group in a meeting where a consensus is obtained. I do not believe this group feels that the conference or meeting method is any way to settle biological problems. Groups meeting for such purposes are usually composed of nonbiologists. Group discussions without data can never produce good criteria. Water cannot rise above its source. There has been too much talk, too many meetings, too much money spent on this unproductive procedure. We must have research to assemble the needed facts. Meetings, arguments, and the seeking of a consensus should be directed to determining what use or uses will be given preference in a given stream in view of the local situation, and the requirements for each use.

I agree completely with Mr. Stein that discussion is no way to set water quality criteria. I cannot agree with him, however, when he says we do not need criteria, that we should go out on the stream, determine what is wrong, and correct it. How can we determine what is wrong if we do not know definitely what is required?

Let us look at the problem from another point of view. The time has now passed, in many sections of our country, when we can afford the luxury of using water only once. Our future fresh-water needs can be met only by re-use. Effective and efficient re-use means that each user must return the used water to the stream in such condition that it is suitable for another use. To do this economically and efficiently, he must know the quality of water required for the next desired use. Gentlemen, we do not know definitely the water quality required for all uses. To put it bluntly, in many instances we do not know our objectives in pollution abatement. Neither do we know definitely our objectives for waste treatment. Without this knowledge, how can we detect and evaluate pollution concisely? We've had the cart before the horse for too long. For too long we have been making field observations; determining BOD, conductivity, etc.; and analyzing water samples for a few materials for which we have field or well-tested analytical procedures. What do we do with these data? How can we apply them? We are at a loss. We put the material in a cubbyhole.

Let me illustrate. Recently I had a letter from a man in Ohio. He said he'd had a phenol problem. He had found phenol in the body of fish. What does he do with the data? I told him we have had other problems like this, come on down, and let us get down to brass tacks and discuss the essentials. How much phenol is harmful? What is the relation between the amount of phenol in the water and the amount found in the fish? Phenol data are often collected because we have an analytical method for phenol. In the past, much data have been collected only because it was customary. There was no definite idea about how the data would be used. It is past time to stop this busy work. Analyses to determine the amounts of materials present have little meaning if we do not know the levels that are harmful. When these levels are known, however, these analyses are meaningful.

We must know the water quality required for each use. This will take research and time and, of course, we can't get the answers all at once. We must have some answers, however, before we can determine what waste treatment is needed. At present many waste treatment requirements are based on opinion. Since opinions differ, requirements differ or are changed as administrators change.

This group is here for the purpose of summarizing our knowledge on water quality criteria. Your program shows the approach we are taking; we're not just discussing criteria, we're not coming together to decide in committee what criteria should be. The program deals with the environmental requirements of aquatic life. This is because water quality criteria for the protection of aquatic life must be based on an adequate knowledge of the requirements of those organisms we intend to protect. If you'll look through your program, you will see that we are dealing with the requirements for the various groups of organisms.

In the setting of criteria we must be governed by the need; that is, the quality of water required for a specific use. When the scientist determines or suggests water quality criteria, he must pay no attention to economics. Let me illustrate. If you were making ball bearings and you were told these must have a certain diameter with a tolerance of 0.001 inch, would you be saving money if you reduced the cost of production by using a tolerance of 0.1 inch? The operation would cost less, but you would be wasting time since the product wouldn't meet the need. The same thing applies to water quality criteria; they must meet the need — insure the quality of water required — or they are useless. Economics enter the picture when the proper authorities decide what use or uses will be given preference on a certain stream. The criteria must define definitely the quality of water required for the use in question.

Water quality criteria have definite uses and values for meeting the pollution problem. They enable us to detect and evaluate pollution. They define our objectives in pollution abatement and waste treatment. They supply definite information on what is needed and provide information that will allow the general public to be a more effective force in pollution abatement. If carefully documented and adequately explained, they should result in more uniform regulations. This will reduce the practice of one locality bidding against another for a desired industry and making concessions at the expense of the aquatic resource.

Criteria for all water uses will be the most important and the strongest tool we can use in legal procedures for the abatement of pollution. They will define the objectives that must be met. With our present lack of knowledge many enforcement actions result in long agruments about what is or is not harmful, what levels are or are not toxic, and what effects are or are not significant. In such a situation, short-term bioassay tests can be used against the public interests. The defense may make a bioassay with resistant adult fish, using in the test solution a concentration of waste comparable to that in the receiving stream. The fish are taken out just before they die and nothing is said about their condition at the end of the test. Then the defense attorney presents the data and asks, "Can fish live for x hours at a concentration comparable to that in the receiving stream? Yes or no." You answer "yes" since they did live, and, without knowledge of the concentrations of the wastes that are safe under conditions of continuous exposure, you cannot establish that such levels are not satisfactory for the survival of the species. We must realize that environmental conditions that the adult may resist for even long periods may be entirely unsatisfactory for the survival of the species. We are not interested in just the lethal effect. We are interested in levels that are not harmful and that do not interfere with productivity. If we have well-documented criteria, and if they are accepted, we will be in a much stronger position in legal actions to abate pollution. Then the question would be: Are these conditions, as defined by the criteria, being met? If they are not, pollution exists and there is a violation. I am convinced that criteria can serve as a firm basis for legal action when it is required.

Surveys to determine if pollution exists become meaningful when the quality of water required for a given use is known. When safe levels of toxicants are known, chemical analyses have meaning. Chemical analyses then become a tool to determine compliance. There is a real need to meet problems in this way. The application of criteria and the enforcement of regulations are not in the field of the scientist. It is his duty to determine the facts and present the needed information. This seminar has as its purposes the summarization of present knowledge of the environmental requirements of aquatic organisms, the presentation of problems, the description of new methods and approaches, the exciting of interest, the exchange of information, and the promotion of good will and cooperation both nationally and internationally. It is my sincere hope that it will be of value to you.

Mr. Weston

Dr. Tarzwell, as a consultant, I can't help but agree with the technical aspects of the problem that you have raised, and certainly you must have the technical know-how. There are two big factors we haven't talked about here today that are of vital importance — one is social acceptance and the other is social economics. Maybe these are coined words, but I'll use these particular terms. I'd like to give as an illustration a situation on a stream in a Pennsylvania area in which we have had considerable complaint concerning the froth, the suds, and the toxicity to aquatic life caused by detergents used by housewives. The housewives have come to public meetings and have indicated their indignation that such a situation should exist. It was pointed out to them that their current sewage treatment facilities are incapable of removing the synthetic detergents from the wastes, but that if they as housewives would use soap — common old Ivory soap or naphtha soap — instead of the synthetic detergents they would no longer have that condition in their streams. Gentlemen, today they still have suds in that stream. The housewives would not revert to the soap. Here we have a matter of acceptance. As to economics, they will not supply the funds so that the sewage treatment plant can remove the synthetic detergents. We know how they can be removed today, but the public will not raise the funds needed to remove them. So, I think in addition to technology you've got to have social acceptance and a respect for social economics.

Mr. Kimball

May I repeat what I said before in connection with water quality criteria problems. It's my feeling that there is no water pollution problem too great for our scientific teams to solve, and that the standards so obtained should be accepted by the people as essential to clean water. Sufficient time and funds should be made available to these teams to do the kind of job that needs to be done. Mr. Adams has stated that detergents can be removed from effluent. Maybe Murray or somebody else can tell us how much public funds have been made available to find out whether or not this can be done economically. If we're going to finance pollution abatement programs solely with funds obtained from bond issues, we never will get the job done. Our present efforts are far from achieving desired results. What is the answer?

Mr. Stein

First, we do have funds that we could use for research on detergents but we're not doing this. I commend to you, however, one of Milt Adams' suggestions that a manufacturer be not permitted to put out a product that is going to create a problem unless he comes up with a solution to it. This may be something to consider. West Germany, you know, banned the use of detergents. For the benefit of the foreign representatives we have here, I think we should point out that we have a system of justice in this country that operates slightly differently from the courts in other countries or from procedures they may have witnessed on movie or TV screens. I doubt that there are very many cases where the judge will not permit an expert to give his full views, but hold him to yes or no. I haven't experienced that. By and large, I think we get a full hearing in pollution cases.

Secondly, I don't think we're losing cases in court because of not having scientific or biological evidence. The cases just aren't brought into court, because of the pressure. I think the gentleman from Canada raised the point of biological evidence. Possibly he missed the essential point or maybe he doesn't have these conditions in his country, but in large segments of the national program and in state after state in this country we have seen instances where administrators in charge of the program for developing criteria, standards, and classification have gone on for years and have not cleaned up a drop of pollution. I agree there is necessarily no dichotomy between them; the practical day-to-day work and the basic scientific work can both go on at one time. Milt Adams has a distinguished record in Michigan he can be proud of. He has that distinguished record, I submit, precisely because he went out on a case-by-case basis and fought to abate pollution in his 32-year tenure in office. He did not, as some state administrators have done, wait for 15 or 20 years classifying streams, getting at the legal battles of classifying streams, and not cleaning up a bit of pollution. We have more data and more research coming out of Milt Adams' operation in Michigan than we have in many of the other jurisdictions that have adopted the other course.

Mr. Kimball

Then, in essence, you're saying that the problem is not one of establishing standards of water quality, but rather one of selling the public, pollution abatement administrators, law enforcement officers, and judges on an action program. Through political pressure, water polluters have been able actually to prevent enforcement cases from being consummated. This has been the reason for lack of adequate pollution abatement rather than inadequate water quality standards. Is that what you're saying?

Mr. Stein

Yes, that's largely what I'm saying. I'm also saying that Tarzwell and any of his compatriots can go into a particular area and do exactly what he says — throw out the substances that are interfering with the normal use of the water as far as the biology is concerned. If we proceed on that basis, we're going to have a cleaned-up area. I also submit that by going into area after area, finding out what is interfering with the legitimate water use from the point of view of a biologist, presenting a description of that, an analysis of that, and the facts of that, we will be much farther along than if we have these cozy little sessions to decide what number we should put on a concentration that will be acceptable in one stream or another, because what will be adequate in a swift-moving stream in the Pacific Northwest won't be applicable in the Southwest. We have many, many streams in this country that are, as we say (in law) sui generis — that's pig latin for saying unique, in, of, or to themselves. I think the time has come when we have to hit this on a case-by-case basis and get ourselves the building blocks, rather than go down the path you have seen many of these agencies take, frittering away their time, their resources, and their energies in maintaining an allowable limit of how much we can possiblly dump into a stream.

ENVIRONMENTAL REQUIREMENTS OF PLANKTON ALGAE AND THEIR EFFECTS ON WATER QUALITY

G. E. Fogg, Chairman*

DIATOMS AND THEIR PHYSICO-CHEMICAL ENVIRONMENT

Charles W. Reimer†

The primary purpose of this paper is to present some measurements of the habitats in which five selected species of diatoms have been found, and to assemble and comment on some of the ecological remarks made about these species.

Much of the relative value from such a study rests on the reasonable assurance that the information gathered actually refers to the same species. Many diatom species are similar in morphological appearance and it is my opinion that considerable misapplied ecological information is available in the literature under a single species name which actually applies to more than one taxon. In an attempt to minimize this possibility, the following relatively well-defined species have been selected: *Melosira varians* C. A. Ag., *Nitzschia amphibia* Grun., *Navicula confervacea* Kütz., *Cymbella tumida* (Breb.) V. H., and *Navicula ingenua* Hust. These species have rather distinctive valve features and, with the possible exception of *N. amphibia*, do not seem to belong to any such series of closely related taxa. This should increase the probability that the information gathered here does refer to the species discussed. The first four species are rather widespread both in this country and throughout the world, and thus a larger number of observations are at hand for comparison.

No attempt was made to cover all the published literature on this subject, but rather to include those larger works which appeared to have more extensive physico-chemical data. Chemical ranges compiled from the literature were from the following sources: Bourrelly and Manguin (1952), Cholnoky (1960) (1962), Foged (1948) (1949) (1951) (1953), Hustedt (1937) (1938a) (1938b) (1939) (1943) (1957), Kolbe (1927), Niessen (1956), Patrick and Freese (1961), Scheele (1952), Schroeder (1939), and Sovereign (1958). Other ecological notes and comments were compiled from the works of other authors listed in the References.

Ecological categories used by other authors to describe some of the physical and chemical tolerances or preferences are mentioned here as they have been applied to each species.

Our own chemical and distribution data are based on surveys carried out by members of the Limnology Department under the direction of Dr. Ruth Patrick. One series of data was processed in a computer using data from 41 different streams and rivers in the United States and bracketed upper and lower physico-chemical ranges. A second series (from 59 areas) was assembled manually from data on the following rivers and creeks: the San Joaquin (California), the Potomac (Maryland), the Tennessee (Tennessee), the Ottawa (Ohio), the Auglaize (Ohio), the Savannah (South Carolina-Georgia), Lower Three Runs Creek (South Carolina), the Schuylkill (Pennsylvania), and Assunpink Creek (New Jersey).

Although the species considered in this paper cannot be considered truly planktonic, they are diatoms which do occur from time to time in the seston of streams and rivers, sometimes in appreciable numbers. Thus, a study of the suspended organisms in a stream can very possibly involve these species.

Melosira varians C.A. Ag.

Table 1 shows the ranges of chemical conditions under which *M. varians* has been observed. For a sample to be included in this tabulation the species must have occurred at least six times at a minimum count of 8,000 diatoms.

For each sample there were accompanying chemical analyses of water collected at the same time as the diatoms. Thus, for example, the number 132 in the pH line of Table 1 represents 132 composited diatom samples in each of which 8,000 of the diatoms were present.

The magnitude of variation in nearly every case is great and might suggest that this diatom is insensitive to changes in any of these factors. Since much of the ecological terminology involves such statements as "relative numbers," "best development," "massive growth," and the like, it seemed advisable to assemble the same kind of data in sample percentage groupings. In Table 2 the percentage occurrence of *M. varians* plus the number of samples within the percentage range is given.

In most cases the greater physico-chemical ranges are to be found where the diatom showed a frequency of less than 1 percent. At higher frequencies these ranges narrowed. These narrower ranges can hardly be considered as more reliable or optimal for the species merely because of increased numbers in the samples since the number of such observations is very low, a factor which seems not always to have been considered in classifying the physico-chemical preferences of a species.

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Table 1. PHYSICO-CHEMICAL DATA (COMPUTER) FOR *MELOSIRA VARIANS* C.A. AG. (00095)

Factors	Ranges	No. of samples
pH	<3.0 to 8.0 - 9.0	132
Temperature, °C	<12.0 to >40.0	128
Total hardness (as $CaCO_3$), ppm	<10.0 to 1000 - 5000	134
PO_4, ppm	<0.001 to 5.0 - 10.0	127
NO_3 (as N), ppm	<0.007 to 1.5 - 3.0	130
NH_3 (as N), ppm	<0.001 to 10.0 -20.0	130
Alkalinity (M. O.) (as $CaCO_3$), ppm	<10.0 to 200.0 - 550.0	134
SiO_2, ppm	<1.0 to> 48.0	121
Fe (soluble), ppm	<0.01 to 1.0 - 5.0	91
Cl, ppm	<3.0 to 2500 - 10,000	134
SO_4, ppm	<10.0 to 240 - 480	118

Table 2. PERCENTAGE FREQUENCY OF PHYSICO-CHEMICAL DATA FOR *MELOSIRA VARIANS* AG.

Frequency, percentage	No. of samples	pH	Temp., °C	Hardness (as $CaCO_3$), ppm	PO_4, ppm	NO_3, ppm N	NH_3, ppm N	Alkalinity (M. O.) (as $CaCO_3$), ppm
<1	27	6.0-8.3	10-32	64-522	0.002-2.7	0.00-4.92	0.00-43.4	22-244
1	3	7.4-7.8	20-30	75-192	0.072-0.143	0.052-0.427	0.008-0.632	73-80
2	7	6.3-8.4	11-27	77-177	0.029-0.216	0.00-0.873	0.00-0.314	17-109
3	3	7.5-8.3	20-36	86-142	0.004-0.097	0.049-0.426	<0.002-0.478	73-106
5	1	7.5-7.8	20-22	--	--	--	--	--
6	1	7.8	30	184	0.191	0.47	0.270	77
7	1	7.5-7.8	20-22	110-117	0.084-0.089	0.049-0.052	0.071-0.083	73-75
8	1	6.8-7.6	17	57-66	0.094-0.119	0.069-0.120	0.494-0.540	52-53
10	1	8.2	31	142	0.010	0.57	<0.001	108
11	3	6.8-8.1	9-28	81-269	0.010-0.525	0.02-1.3	<0.001-0.018	20-193
15	1	6.9-7.5	16-17	65-70	0.073-0.078	0.022-0.069	0.423-0.620	1
20	1	7.5	26	165	0.212	1.4	0.02	49
40	2	7.2-7.7	16-27	65-161	0.073-0.508	0.02-1.5	0.04-0.62	51-57
50	1	7.4	26	166	0.290	1.6	0.02	49
Range		6.0-8.4	9-36	57-522	0.002-2.7	0.00-4.92	0.00-43.4	1-244
Literature		3.0-9.5	0-40	49-712	0.03-0.26	0.2-1.70	0.24-0.41	10-750

Frequency, percentage	No. of samples	Specific conductivity mho	SiO_2, ppm	Fe, ppm	C1, ppm	SO_4, ppm	DO, % sat.
<1	27	1.2-9.8x10^{-4}	0.6-10	<0.001-0.54	6-1470	24-452	10-100
1	3	1.5-5.8x10^{-4}	3.4-9.3	<0.002	14-192	91-189	40-90
2	7	1.39-3.46x10^{-4}	0.7-11.4	<0.002-0.14	14-306	23-61	70-90
3	3	1.6-3.8x10^{-4}	3.2-9.3	< 0.002	8-180	25-189	90-100
5	1	--	--	--	876-1470	--	70
6	1	5.8x10^{-4}	7.2	<0.002	27	140	80
7	1	--	8.7-9.3	<0.002	168-192	91-189	90
8	1	--	12.7-12.9	<0.002-<0.020	11-17	23-25	90
10	1	3.9x10^{-4}	3.0	<0.001	12	57	90
11	3	1.4-5.9x10^{-4}	3.0-7.0	0.001-0.089	13-23	45-152	50-80
15	1	--	12.2-12.7	0.002-0.021	11-20	20-32	100
20	1	4.5x10^{-4}	9.0	<0.002	17	149	90
40	2	4.4x10^{-4}	9.0-13.0	<0.002	21	20-160	80
50	1	4.6x10^{-4}	9.0	<0.002	14	155	80
Range		1.2x9.8x10^{-4}	0.6-12.9	<0.001-0.54	6-1470	20-452	10-100
Literature		2.0x10^{-4}-2.0x10^{-5}	<1->6	Total <2.5->10	16-2000	--	0-100+

It is, nevertheless, useful to have recorded such ranges as a starting point. When we have assembled as much water chemistry data for massive-growth occurrences as we have for small-number occurrences, we might be in a much better position to delimit the conditions for "massive growth" and perhaps to decide whether or not this coincides with the "range of best development."

Table 2 compares the chemical ranges in which *M. varians* has been found in our various stream surveys, with ranges compiled from 35 works of other investigators.

Of the ranges given there is no substantial disagreement between our survey data and the recordings of others, though in some cases our ranges are broader (e.g., SiO_2 and PO_4).

The following terms have been used by various authors to describe the apparent physical and chemical preferences of this species.

Alkaliphil (occurring around pH 7, with principal distribution at a pH of over 7) -- The few large populations of this species observed here did occur at a pH range slightly above 7. Thus, greatest growth of the species has been recorded in the range wher others have reported best growth. I would no however, be completely satisfied to report that *M. varians* appears as a true alkaliphil in this country until: (1) many more massive occurrences have been recorded with accompanying water quality data and (2) many more populations from acid streams and rivers have been observed.

Oligohalobe (salt content not necessary, able to survive to about 5,000 ppm) -- The upper range of chloride tolerance found from our surveys was somewhat lower than 5,000 ppm.

Euryoxybiont (tolerant of a wide range of oxygen content) -- Table 2 shows presence at a rather wide range of oxygen saturation.

Beta-mesosaprobe (living in and indicative of the pollution zone in which ammonia compounds of the fatty acids are found and in which further oxidation takes place) -- Until a more precise chemical definition for this zone can be made, it is not possible to decide how indicative this species is of this zone.

Saprophyte (a diatom assuming the function of a saprobiont, i.e., massive in polluted waters, appearing as if restricted to this habitat) -- Hustedt (1957) states that *M. varians*, when appearing in massive amounts, can be considered a saprophyte, not necessarily because of a preference for this organically enriched habitat, but because of its euryplastic nature (wide tolerance range) coupled with the disappearance of other stenoplastic (narrow tolerance) species. Palmer (1959) lists the alga as an indicator of polluted conditions, but makes no mention of frequency as an indicator. Our data show appreciable NO_3 content when frequencies over 15 percent are present. In these particular cases the total diatom species numbers were depressed.

Eutrophic (waters with considerable amounts of nutrients) -- Such a category is indicated by the data. There may be some correlation with this designation and Sovereign's 1958 statement that he found *M. varians* rare in the mountains where waters are commonly oligotrophic and common in the lowlands where mesotrophic to eutrophic waters are much more common.

Current-indifferent (no preference for fast- or slow-flowing water) -- Field observations of my own and habitat designations for many collections of this species confirm this category.

Eurythermal -- Temperature ranges are broad enough that this diatom can be considered eurythermal.

Maximum development is considered by Scheele (1952) to occur in winter. Liebmann (1951) and Schroeder (1939) report a maximum in summer. I have seen this species during all seasons, but our few high-frequency samples (collected in summer) are insufficient to label any particular season most suitable for maximum growth. In this case it is probable that season is important as it applies to one river system in one area, but that generalizations applied to large climatic and geographic areas are not meaningful.

Nitzschia amphibia Grun.

The data for this and the remaining species were taken in the same manner as described for *M. varians*; therefore, no further general comments need be made regarding collection or presentation of data, Tables 3 and 4.

Upper ranges for sulphates (511 ppm) and phophates (ca. 8 ppm) are considerably higher in our survey data than recorded by other authors. On the other hand, hardness figures (712 ppm) of other authors exceed the upper range of our survey data.

The spectrum is again broad for this widespread species in the categories considered. The following ecological terms have been used in connection with *N. amphibia*.

Alkaliphil -- All of the larger populations of *N. amphibia* were found in waters with a pH above 7.0. Only in the less than 1 percent groupings was the range lower (pH 6.3). Our figures are in agreement with this category although the same comment made for *M. varians* in the discussion of pH is applicable here. Cholnoky (1962) claims the "optimum" pH for this species is over 8.0.

Oligohalobe -- A chloride content of 1,470 ppm was recorded when this species was present in less than 1 percent of the samples in a collection from the San Joaquin River. The higher percentage occurrences were in water with a chloride content of less than 300 ppm.

Haloxene (an accidental guest, a "stranger" in salty-water communities) -- Scheele (1952) prefers

Table 3. PHYSICO-CHEMICAL DATA (COMPUTER) FOR *NITZSCHIA AMPHIBIA* GRUN. (00141)

Factors	Ranges	No. of samples
pH	7.5 - 8.0 to 8.0 - 9.0	32
Temperature, °C	<12.0 to 30.0 - 35.0	80
Tl. hardness (as $CaCO_3$), ppm	<50.0 to 500.0 - 1000.0	82
PO_4, ppm	<0.001 to 5.0 - 10.0	79
NO_3 (as N), ppm	<0.03 to 3.0 - 7.0	81
NH_3 (as N), ppm	<0.001 to 10.0 - 20.0	81
Alkalinity (M. O.) (as $CaCO_3$), ppm	<20.0 to 200.0 - 550.0	82
SiO_2, ppm	<3.0 to 12.0 - 48.0	72
Fe (soluble), ppm	<0.01 to 0.4 - 0.7	37
Cl, ppm	<3.0 to 500.0 - 2500.0	82
SO_4, ppm	<10.0 to 240.0 - 480.0	71

Table 4. PERCENTAGE FREQUENCY OF PHYSICO-CHEMICAL DATA FOR *NITZSCHIA AMPHIBIA* GRUN.

Frequency, percentage	No. of samples	pH	Temp., °C	Hardness (as $CaCO_3$), ppm	PO_4, ppm	NO_3, ppm N	NH_3, ppm N	Alkalinity (M. O.) (as $CaCO_3$), ppm
<1	35	6.3-8.4	10-36	57-416	.004-2.7	0-4.9	0-4.9	21-243
1	2	7.5-7.8	22-26	93-168	.092	0-.83	0-.03	64
2	3	7.4-7.7	22-28	65-166	.133-.290	.080-1.58	.022-1.25	49-70
3	4	7.5-8.0	25-30	64-269	.035-.482	.018-.47	.018-.50	66-193
7	1	7.4	26	172	--	.03	0.03	--
10	2	7.5-7.7	26-27	161-165	.20-.51	1.41-1.52	.025-.042	49-51
11	1	7.3	28	405	7.98	.508	.181	177
Range		6.3-8.4	10-36	57-416	.004-7.98	0-1.58	0-4.9	21-243
Literature		2.0 - 9.0	2-40	35-712	.060	23-170	--	10-750

Frequency, percentage	No. of samples	Specific conductivity mho	SiO_2, ppm	Fe, ppm	Cl, ppm	SO_4, ppm	DO, % sat.
<1	35	$1.4\text{-}8.6 \times 10^{-4}$	0.7-12.9	<0.001-0.39	6-1470	20-511	40-100
1	2	1.8×10^{-4}	0.6-7.9	0.001-0.01	6-56	23-25	90-100+
2	3	$1.4\text{-}4.6 \times 10^{-4}$	2.3-9.3	<0.001-0.54	11-14	155	80-92
3	4	$1.7\text{-}5.9 \times 10^{-4}$	0.6-7.2	0.0-0.09	10-23	40-152	50-82
7	1	--	0.6	0.00	50	63	84
10	2	$4.4\text{-}4.5 \times 10^{-4}$	9.5-9.8	<0.002	17-21	149-160	80-90
11	1	--	--	0.044	94	290	100
Range		$1.4\text{-}8.6 \times 10^{-4}$	0.6-12.9	0.0-0.54	6-1470	20-511	40-100
Literature		$2 \times 10^{-5}\text{-}2 \times 10^{-4}$	0.4-6.0	Total 0.4->10	13-2000	5-6	0-100

to call it indifferent to slight salt concentrations rather than haloxene. Whatever the case, and the choice would be difficult, this species is recorded here only in very low frequencies where salt concentrations are above 100 ppm.

Mesooxybiont (tolerant of intermediate concentrations of oxygen) -- We have observed this species where oxygen concentrations are between 40 and 100 percent of saturation. Data of Foged (1948), however, indicate minimum range occurrence at 0 percent oxygen, which would place it in the category of euryoxybiont.

Beta-mesosaprobe -- We have no correlative observations on large populations (above 11 percent) of this species with water quality. The ranges available do indicate presence in waters containing relatively large amounts of NO_3, PO_4, and in lesser amounts of oxygen.

Current-indifferent -- This diatom has also been collected in both fast and slow water in this country.

Euryzonous (occurring at various altitudes) — Cholnoky and others object to the use of altitude as an ecological factor important in diatom distribution. It is true that most mountain streams are oligotrophic and most lowland streams are mesotrophic to eutrophic, aspects of which may call forth different biological expressions not relating to height above sea level. Nevertheless, we should be careful about discarding altitude altogether as a factor unless we wish to ignore pressure, light intensity and quality, differences in gas exchange, etc., as contributing factors. The situation with pH may well be analogous to altitude. In many instances the well-used factor of pH is probably only important as it affects other critical physico-chemical aspects of the environment.

Another factor not commonly mentioned for diatom species is that of light. This species is considered by Scheele (1952) (1954) and Hustedt (1937) as characteristic of the *dysphotic* zone. This is probably to say that it is found under a wide range of light intensities and has a low compensation point, since we have often found it on substrates in clear water near the surface. Scheele (1954) has also reported it from surface samples.

Table 5. PHYSICO-CHEMICAL DATA (COMPUTER) FOR *NAVICULA CONFERVACEA* KÜTZ. (00104)

Factors	Ranges	No. of samples
pH	<7.5 to 8.0 - 9.0	36
Temperature, °C	<17.0 to 28.0 - 35.0	30
T1. hardness (as $CaCO_3$), ppm	<50 to 500 - 1000	35
PO_4, ppm	<0.001 to 0.5 - 5.0	34
NO_3 (as N), ppm	<0.013 to 0.7 - 1.5	35
NH_3 (as N), ppm	<0.009 to 1.0 - 10.0	35
Alkalinity (M. O.) (as $CaCO_3$), ppm	<10.0 to 100.0 - 200.0	35
SiO_2, ppm	<6.0 to 6.0 - 43.0	35
Fe (soluble), ppm	<0.01 to 1.0 - 5.0	31
C1, ppm	<3.0 to 2500 - 10,000	35
SO_4, ppm	<10.0 to 480 - 960	35

Navicula confervacea Kütz.

There are fewer comparative data for this species than for the first two diatom species (Table 5). Likewise, our own samples are fewer. For some chemicals no ranges of tolerance were found in the literature used.

The one high-frequency occurrence was from a tributary of the Savannah River, Lower Three-Runs Creek. This is a rather well-aerated, dystrophic, soft-water stream with very little organic material and a moderate to swift current.

Cholnoky (1958a) (1960) considers this diatom to be an indicator of "succession," one which can withstand gross changes in nitrogen content. He states that its frequent occurrence (usually in association with *Gomphonema parvulum* and *Navicula seminulum* in the Jukskei River) is a sign of "unstable" conditions. He also considers that it can be frequently associated with an increase in nitrogen content when it occurs in large numbers.

Our figures show it did occur once at an appreciably high NO_3 concentration (Table 6). Hustedt (1937) found it "massive" in 21 of 108 samples and states that whenever it occurs in masses it is to be considered as beta-mesosaprobic.

"Maximum" development is recorded by Bourrelly and Manguin (1952) at a pH of 6.0 to 7.5 and by Hustedt (1937) at a pH of 7.0 to 7.5. The pH range of our higher frequencies was well above 7.0. Hustedt's data would indicate that this species is an alkaliphil. Bourrelly and Manguin's data would cast doubt on this designation. Perhaps it would better be classified as indifferent.

With respect to temperature this diatom should be considered as *eurythermal*.

Cymbella tumida (Breb.) V.H.

Computer analysis ranges for this taxon are given in Table 7, and selected frequency ranges are found in Table 8. Relatively broad ranges again are indicated for most factors. This is also indicated by the available data from the literature. Several ranges are recorded here for individual chemical factors not found in other reports.

High frequencies of this species have not been recorded by us from streams and rivers in this country where the chemistry of the water has been taken. It does occur sometimes in large numbers in an individual collection, but it has not been observed in great amounts throughout a large area of any river section investigated so far. Scheele (1952) also found it only infrequently in the Fulda River. Since it is a large cell its presence in smaller percentages may be of greater significance than is true of the smaller species in reflecting conditions of the biotope (cf., Hustedt, 1957, p. 187). Other comments applicable to this species follow.

Table 6. PERCENTAGE FREQUENCY OF PHYSICO-CHEMICAL DATA FOR *NAVICULA CONFERVACEA* KÜTZ.

Frequency, percentage	No. of samples	pH	Temp., °C	Hardness (as $CaCO_3$), ppm	PO_4, ppm	NO_3, ppm N	NH_3, ppm N	Alkalinity (M. O.) (as $CaCO_3$), ppm
<1	11	6.3-8.3	8-36	65-387	0.002-2.06	0.022-2.81	<0.001-0.62	2-200
2	1	7.7-8.4	26-34	91-105	0.047-0.216	0.234-0.357	0.018-0.102	93-103
6	1	7.8	30	184	0.191	0.470	0.270	77
13	1	7.5	26	165	0.212	1.410	0.025	49
38	1	7.3	22	41	0.058	0.146	--	--
Range		6.3-8.4	8-36	41-387	0.002-2.06	0.022-2.81	0.001-0.62	2-200
Literature		4.0-8.2	15-40	--	--	--	--	10-750

Frequency, percentage	No. of samples	Specific conductivity mho	SiO_2, ppm	Fe, ppm	C1, ppm	SO_4, ppm	DO, % sat.
<1	11	1.39-5.8x10^{-4}	3.0-12.7	0.001-0.089	11-180	20-260	40-100
2	1	2.72-3.46x10^{-4}	5.5	--	130-146	33-53	--
6	1	5.8x10^{-4}	7.2	<0.002	27	140	80
13	1	4.5x10^{-4}	9.5	<0.002	17	149	90
38	1	8.7x10^{-5}	11.1	0.116	5	1	85
Range		8.7x10^{-5}-5.8x10^{-4}	3.0-12.7	0.001-0.116	5-180	1-260	40-100

Table 7. PHYSICO-CHEMICAL DATA (COMPUTER) FOR *CYMBELLA TUMIDA* (BREB.) V. H. (00051)

Factors	Ranges	No. of samples
pH	7.5 - 8.0 to 8.0 - 9.0	65
Temperature, °C	<12.0 to 30.0 - 35.0	64
T1. hardness (as $CaCO_3$), ppm	<50.0 to 1000 - 5000	65
PO_4	<0.005 to 0.5 - 5.0	65
NO_3 # (as N), ppm	<0.007 to 0.7 - 1.5	64
NH_3 # (as N), ppm	<0.001 to 1.0 - 10.0	64
Alkalinity (M. O.) (as $CaCO_3$), ppm	<30.0 to 150.0 - 550.0	65
SiO_2, ppm	<3.0 to 12.0 - 48.0	59
Fe (soluble), ppm	<0.01 to 0.4 - 0.7	46
C1, ppm	<0.5 to 2500 - 10,000	65
SO_4, ppm	<10.0 to 90 - 240	58

Table 8. PERCENTAGE FREQUENCY OF PHYSICO-CHEMICAL DATA FOR *CYMBELLA TUMIDA* (BREB.) V. H.

Frequency, percentage	No. of samples	pH	Temp., °C	Hardness (as $CaCO_3$), ppm	PO_4, ppm	NO_3, ppm N	NH_3, ppm N	Alkalinity (M. O.) (as $CaCO_3$), ppm
<1	9	7.3-8.4	20-29	77-159	0.009-0.304	0.194-0.746	<0.002-0.314	64-109
3	3	7.4	26-27	117-176	--	0.00-0.03	0.00-0.03	--
4	1	7.6	26	166	--	00	00	--
8	1	7.8	26	168	--	00	00	--
Range		7.3-8.4	20-29	77-176	0.009-0.304	0.00-0.746	0.00-0.314	64-109
Literature		4.0-8.5	15-35	144-712	--	--	--	10-750

Table 8. PERCENTAGE FREQUENCY OF PHYSICO-CHEMICAL DATA FOR *CYMBELLA TUMIDA* (BREB.) V. H. (CONTINUED)

Frequency, percentage	No. of samples	Specific conductivity mho	SiO_2, ppm	Fe, ppm	Cl, ppm	SO_4, ppm	DO, % Sat.
< 1	9	$1.7-3.7\times10^{-4}$	5.0-8.0	0.0-.013	6-146	22-55	93-100+
3	3	--	0.6-0.7	0.0-.04	32-50	57-63	80-84
4	1	--	0.7	0.0	56	24	88
8	1	--	0.6	0.01	56	23	90
Range		$1.7-3.7\times10^{-4}$	5.0-8.0	0.0-.04	6-146	22-63	80-100+
Literature		--	--	--	22-2000	--	--

Alkalibiont --Foged (1948) considers the species as such but with the recorded pH range of 6.5 to 8.3 given by him it would either have to be an alkaliphil or a pH-indifferent species, if one considered the term alkalibiont in its strict sense. Hustedt (1957) lists it as pH-indifferent. We have no recorded ranges below pH 7 for this species.

Oligohalobe -- Although the computer analysis records chlorides up to a value above 2500 ppm, the selected frequency data show very low chloride readings. Foged's records at 200 ppm show occurrence of the diatom as "rare."

Oligosaprobe -- Although so listed by Hustedt (1957), he remarks that the more frequent occurrence on rotting plants in the vicinity of sewage influxes points to a preference for more markedly *eutrophic* habitats.

Current-indifferent -- Foged (1948) considers the species as such. I have also observed it both in fast-flowing water and in more quiet back-water areas.

Navicula ingenua Hust.

This diatom was identified from this country for the first time in 1958 by Dr. L. G. Livingston of our laboratories. The species was found in a collection series from the Tennessee River made in October of that year. The growth was principally on log surfaces, sometimes intermixed with growths of a fresh-water sponge.

Later, in June 1960, it was recorded in smaller numbers in the Savannah River. In the fall of the same year another collection from Lower Three Runs Creek also contained a small number of this diatom species.

Chemically, the waters in which this species was found are similar, as Table 9 shows. Nitrogen compounds and alkalinity were slightly higher in the Tennessee River. Silicon was higher in the Savannah River, but no importance can be attached to this at present. All three areas at the time of collection showed the following water quality: soft, very low in chlorides, well aerated with moderate to low nitrogen and phosphorus levels, moderately buffered, low levels of sulfate, and a pH not strongly deviating from neutral.

Table 9. PHYSICO-CHEMICAL DATA FOR *NAVICULA INGENUA* HUST.

	Tennessee		South Carolina	
	Sta. 2 (21%)[a]	Sta. 3 (3%)	S. R. (<1%)	L3R (<1%)
pH	7.5	7.7	6.8	6.8
Temperature °C	22	22	24	25
Tl. hardness (as $CaCO_3$), ppm	65-68	64-66	19	37
PO_4, ppm	0.185	0.145	0.15	0.092
NO_3, (as N), ppm	0.380-0.180	0.100-0.040	0.207	0.118
NH_3, (as N), ppm	0.580	0.500	<0.001	0.047
Alkalinity (M. O.) (as $CaCO_3$), ppm	68-69	66-68	18	6
Specific conductivity	1.7×10^{-4}	1.7×10^{-4}	6.5×10^{-5}	1.0×10^{-5}
SiO_2, ppm	2.3	1.9	14.6	6.2
Fe (soluble), ppm	0.36	0.09	0.72	0.53
Cl, ppm	10	10	7	6
SO_4, ppm	--	--	8	10
Dissolved oxygen, %	82	82	83	70

[a] Percentage occurrence of species.

DISCUSSION AND SUMMARY

The foregoing information has given some chemical ranges of tolerance of the five species considered. Some attempt has been made to qualify the species presence and record the chemical characteristics of the waters at given percentage levels.

These figures in no way are intended to reflect the true range for any particular chemical. When one considers all the possibilities for chemical and biotic interaction, it is truly amazing that any general patterns emerge at all.

Of the common species discussed here, mere presence appears to have no direct relationship to water quality changes, at least to those measured. Occurrence in relatively large amounts does show frequent agreement with ecological destinations of others. However, such small numbers of high-frequency occurrences, both in this study and in others, are only additive and do not yet fix physico-chemical boundaries except in a general way.

To use quantity as an indicative factor poses some problems. First is the difficulty of knowing what is meant by "best development." Does this mean simply frequent recurrence of the species under given ranges of conditions?

Niessen, for example, gives the following data for *Nitzschia amphibia* collected in: 3 samples at a pH of 3.5 to 5; 4 samples at 5 to 6; 23 samples at 6 to 7; 29 samples at 7 to 8.5; 2 samples at over 8.5. For the same species Hustedt gives: 1 sample at a pH of 2 to 3; 1 sample at 3 to 4; 6 samples at 4 to 5; 10 samples at 5 to 6; 25 samples at 6 to 7; 177 samples at 7 to 8; 199 samples at over 8.0. Based on these figures *N. amphibia* would be considered an alkaliphil species. It has, to be sure, been found by various workers including myself in alkaliphilous situations and commonly so, but mere totaling of occurrences without knowing whether equal numbers of samples were made under acid conditions or what the year-round picture is cannot lead to definite conclusions.

Perhaps "best development" means occurrence in great numbers. This is fine except that it is sometimes difficult to know just when a species can be called "dominant," "massive," or "quite common." Use of a cell counting technique, although it too has its shortcomings, seems to be somewhat more desirable than an estimation of frequency, even though this estimation is based on several samples from the same habitat (Scheele, 1952).

In most rivers and streams in this country, composited collections from both unpolluted and rather polluted areas seem to yield about the same cell size diversities. Thus percentages of a given species related to a mixed-size population should equate fairly well.

The information presented here in the tables for *M. varians* indicates that about 20 to 40 percent frequency may be high enough by our method to consider the diatom "present in large numbers" and thus some measure or indication of organic enrichment or pollution, since the nitrogen content was higher at the high-percentage occurrences. However, the presence of high amounts of nitrogen does not always indicate large populations of *M. varians.*

There was a less than 1 percent occurrence of this species in the Ottawa River at NO_3 and NH_3 amounts of nearly 5 ppm. At a different location in the same river, the species also had a less than 1 percent frequency at an NH_3 concentration of 43.4 ppm. In the former case, with 5-ppm concentrations of NO_3 and NH_3, there was a high incidence of other species. *Navicula* sp. accounted for 30 percent of the total population, *Nitzschia intermedia* Hantz. for 15 percent, and *N. palea* (Kütz.) W. Sm., 13 percent. The total species present six or more times was 36, a depressed figure for this river section. At the second location in the Ottawa River where the NH_3 concentration was over 43 ppm, *N. intermedia* represented 43 percent and *N. palea* 25 percent of the total population. Total species present six or more times were reduced to 19.

This would indicate that certain species do gain ascendancy in situations with high nitrogen content, but, depending on the available population, the species may be different. This may point to further selectivity under conditions of organic enrichment.

It would seem advisable to continue our attempts to learn what major imbalances or changes in water quality actually call forth in the way of dominancy of individual species, but to accompany these observations with a clear look at the entire population structure, according to species diversity as proposed by Patrick (1949) and Patrick *et al.* (1954), as an equally important and very necessary part of any major change in water quality.

Further, we need to know more about the continuous developmental cycle of individual rivers, the taxonomy of the inhabiting flora and fauna, their fluctuations throughout the year, and their reactions to constant change in physico-chemical factors of the river.

As Fjerdingstad (1950) and Cholnoky (1960) suggest, the magnitude and rapidity of fluctuations may be as important as the maximum - minimum recordings.

Fjerdingstad (1950) states (p. 100) . . "diatoms . . . seem on the whole to be much more sensitive to poisons than the Cyanophyceae. This is an added reason for not regarding the individual diatoms as very suitable indicators of pollution, especially of the order alpha-mesosaprobic and polysaprobic" . . . This would only seem to add support to the use of diatom diversity as an indicator of altered water quality, as proposed by Patrick *et al.* (1954).

This is likewise in line with a remark made by Hustedt (1957, p. 196). He says that the number of saprobic situations in which diatoms develop in great masses is not great, this zone being more characterized by a negative factor, i.e., the absence of the other species characteristic for that water but which are more oxygen-stenoplastic.

Of the factors considered, the most frequently reported for diatoms are pH, temperature, and, to a lesser extent, categories of pollution.

The pH has been considered as one of the most important factors which correlate with diatom occurrences (Hustedt, 1939, 1956). Cholnoky (1960) emphasizes that the magnitude of pH fluctuations is of prime importance and, in this connection, that the buffering capacity should always be considered, since weakly buffered waters have very different diatoms than strongly buffered waters at the same general pH.

The matter of "optimum growth range" or "best development" cannot be decided on data from a few mass populations. The data we have on greater frequencies do show pH, temperature, and other water quality ranges in general agreement with the statements made by others, but, until large numbers of a species in question are observed many more times under the conditions we suppose represent its range of greatest growth, we will do well to use these key-word physico-chemical categories with some caution.

For purposes of indicating a certain level of water quality in a river, it may become an academic question as to what the reasons are for the overdominance of a single species or a few species, that is, whether the diatom species is just "living alone and has had room to spread out," or whether it is really thriving under optimal conditions. Nevertheless I would suggest that the terms "optimum growth conditions" or "range of most suitable growth" be replaced by some such statement as "range of greatest growth" or better still by a relative frequency range.

Temperature ranges are broad for the four common species considered. Few stenothermal species have been reported, the term eurythermal being more applicable to the great bulk of diatom species.

Of the four more commonly occurring diatoms discussed *C. tumida* appears less clearly associated with organic pollution. Our data indicate it can tolerate elevation in nitrogen content, but we have no evidence that would indicate it appears in larger numbers under higher nitrogen concentrations.

The exact role that *Melosira varians, Navicula confervacea,* and *Nitzschia amphibia* may play as indicators of pollution is not yet clear. They are all reported to be variously involved with the presence of elevated nitrogen content. They have been observed in larger numbers than usual when organic enrichment and moderate to heavy pollution are present. On the other hand, even though organic pollution is present the diatom may be present only in very small numbers. In such cases, however, their continued presence in small numbers could be a function of the presence of another slightly more tolerant species.

It is most probable that other diatom species do show much narrower ranges of tolerance and can be more clearly categorized. These more widespread species just discussed have wide ranges of physico-chemical tolerance, making their most efficient range of growth much more difficult to establish.

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THE APPLICATION OF DIATOM ECOLOGY TO WATER POLLUTION AND PURIFICATION

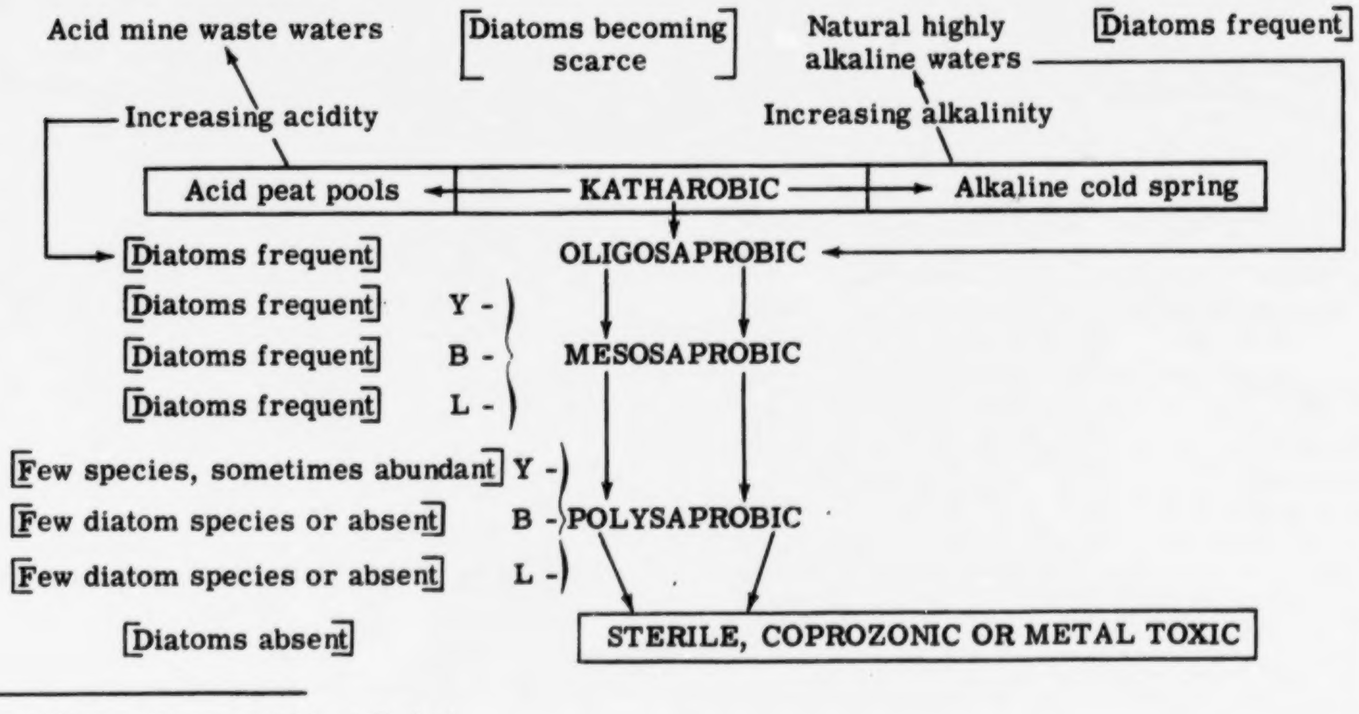
F.E. Round *

Diatoms are the dominant plant organisms in many natural habitats and as such are subtle indicators of the environmental conditions, whilst they themselves modify the habitat.

In an aquatic habitat the micro-organisms occur as a film over all the surfaces and also as a suspension (plankton); the water flows over and through this "filter" of micro-organisms, affecting the composition of the water and at the same time modifying the flora. The "self purification" of polluted waters is certainly enhanced by the growth of such micro-organisms amongst which the diatoms are prominent. The study of the interaction of the diatoms and the waters is a most profitable and important aspect that has perhaps been somewhat neglected in favor of the relationship between floras and degrees of pollution. Neither can be studied advantageously in isolation for they are complementary aspects of a biodynamic system.

It is not sufficient for the algal ecologist to know that a water is polluted because it is chemically or bacteriologically impure; an algal index of pollution is necessary, which is not possible unless there is a standard unpolluted flora to refer to. The penetration of diatoms into polluted waters can then be related to this standard and the whole seen in perspective. The standard can only be the diatom flora of pure natural water, the nearest approach to which in nature is the water of cold springs that is usually alkaline, nutrient rich, organically unpolluted, and at almost constant low temperature (around 9°C.), or the collections of rainwater in small bog pools on moorlands and heaths that are of low nutrient status, highly acid but organically rich. Both these waters exhibit a highly characteristic diatom flora. The former is basically the simplest since the constant flow provides new nutrients, carries away any extracellular and decay products, and may also prevent interaction between species, whilst the latter is complicated by the accumulation of products of assimilation, etc. All other so-called unpolluted waters are complicated by varying degrees of "pollution" by inorganic and/or organic chemicals right through to the extremes of sterility where no diatoms exist. Water more acid than that of peat pools is probably found only in regions receiving acid mine wastes, whereas waters more alkaline than that of cold springs are found naturally in many calcareous regions. From these two types water passes through the well-known series to complete sterility or is maintained at some stage prior to this.

This series can be found in running or standing waters, which adds a considerable problem since distinct floras are found in the two habitats and a species may not have the same tolerance to pollution in each habitat. Although much is known about diatom species distribution, the data are mainly qualitative; although these data are of great value when indicator organisms are considered, they are of less use for the majority of species that occur over a wide range of polluted conditions. On a quantitative, cell number basis, however, most species have a distinct optimum


Acid mine waste waters
[Diatoms becoming scarce]
Natural highly alkaline waters
[Diatoms frequent]
Increasing acidity
Increasing alkalinity
Acid peat pools
KATHAROBIC
Alkaline cold spring
[Diatoms frequent]
OLIGOSAPROBIC
[Diatoms frequent] Y -
[Diatoms frequent] B -
[Diatoms frequent] L -
MESOSAPROBIC
[Few species, sometimes abundant] Y -
[Few diatom species or absent] B -
[Few diatom species or absent] L -
POLYSAPROBIC
[Diatoms absent]
STERILE, COPROZONIC OR METAL TOXIC

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of growth dependent upon the habitat conditions; the accumulation of such data is necessary before any species can be used as an indicator of the degree of pollution. Unfortunately very few detailed studies have been made on any species in nature where it is important that primary quantitative data be collected, or in the laboratory where deductions from studies in the natural habitat can be checked and the effects related to the metabolism of the organism. Three further problems are superimposed on the ecological approach: first is the recognition of the individual microhabitats; secondly, the sampling of these habitats; and thirdly, the clear recognition of the degree of pollution when the diatoms are being used as indicators.

To utilize diatoms in pollution studies, it is necessary to appreciate the range of habitats and the discreteness of each diatom flora within its habitat; otherwise, an impossibly large number of species can be determined, *e.g.*, some 615 species were recorded by Hustedt (1957) in the polluted R. Weser (an indication of the tolerance of diatoms to pollution, although only 17 species were classified as saprophytic). The details of diatom floras in microhabitats are still very incompletely known, as are the tolerances of each species to the gross habitat conditions such as substrata, water flow, temperature, pH, etc. Since diatoms are siliceous organisms, attention has been focused on the supply of silicates in the habitats and relatively slight consideration has been given to the organisms general physiological requirements, metabolic pathways, extracellular products, etc. Finally the intricate nature of the siliceous cell wall has led to an overwhelming concentration on their taxonomy.

One of the simplest and best documented groups involving diatoms is the phytoplankton community floating in the water. It is, however, of little use in flowing waters (Fjerdingstad, 1960; Patrick, 1957) except where flow is reduced *e.g.* by tidal pressure or damming, or in very large slow-flowing rivers, since lenitic phytoplankton is merely a collection of casual species from the drainage basin. Hustedt (1957) has been able to trace the gradual increase in pollution in the R. Weser over the last 50 years from the diatom records. The occurrence of *Nitzschia acicularis*, *Staphanodiscus hantzschii*, and *Melosira varians* is an indication of organic pollution ("Saprophytic species"); yet these are all common in waters which, although admittedly rich in organic matter, could not be termed polluted. Both the *Nitzschia* and the *Melosira* species are also extremely abundant on sediments and are merely another example of the accumulation of littoral species in the plankton under certain conditions. *Stephanodiscus* is a frequent diatom of waterworks storage reservoirs and can hardly be an indicator of pollution. On the other hand, the Weser in the region of Bremen is polluted, and the list of planktonic species suggests that the combination of large cell numbers of *Cyclotella*, *Stephanodiscus* and *Thalassiosira* together with a large number of species of *Nitzschia* is characteristic of this polluted river. Species of the above centric genera are rarely found, however, when submerged slides are used. A good example of lake phytoplankton populations is that reported by Jarnefelt (1961) who showed that pollution of a lake from a small town favored the growth of *Asterionella gracillima*, *Cyclotella stelligera*, *Fragilaria crotonensis*, *Melosira granulata*, *M. islandica*, and *Synedra berolinensis*, whilst *Rhizosolenia eriensis* appeared only in samples from the town polluted region and not at all in samples from the purer lake waters above the pollution or below in the chemically polluted region. Other species also show a slight enhancement of growth in this region, and although it is true that "no species is *adapted* to living in polluted conditions which are the products of civilization and so very much younger than is any species" (Hynes, 1960), it is equally true that all so-called natural populations are modified to some extent by "organic pollution" from the moment that heterotrophic, excretory organisms appear in the habitat.

Chemical pollution by man-made chemicals is another matter. The danger in Jarnefelt's analysis is that species carried into more polluted areas are still recorded although they may be dying off;* e.g., it seems unlikely that *Fragilaria crotonensis* is equally adapted to the pollution from sulphite wastes in the zone below the town whilst *Rhizosolenia eriensis* is not. The presence of the former in the samples and the absence of the latter may merely reflect different sedimentation rates, for in the less polluted waters below the zone of maximum pollution from sulphite, in the so-called zone of "intensive photosynthetic purification," *Fragilaria* and other algae are less abundant. An alternative explanation is that the combination of organic and chemical pollution allows some of the organisms that are sensitive to chemicals to grow, whilst beyond this where the chemical pollution is accompanied by decreasing organic pollution these same organisms are eliminated. If this latter hypothesis is correct, then experimentation with combinations of pollutants may lead to methods for enhancing self-purification, since in the zone of sulphite pollution no diatoms were characteristic or achieved greater numbers than elsewhere.

In many Swedish rivers there is a characteristic growth of *Frustulia rhomboides* and *Synedra ulna* below regions of sulphite discharge from wood pulp factories. *F. rhomboides* is an acid-loving species, which may account for its occurrence in these habitats, but it also has its cells embedded in mucilage that may act as a buffer between the medium and the cell (cf. *Staurastrum* below). In S. Africa it has been shown to be present at pH below 3.0 in acid-polluted streams. This is a species common on sediments, coating stones and water plants, etc., and it may prove to be the only true diatom component of such waters since plankton is obviously not adapted to such conditions.

The other freely motile community is that on the sediments (epipelic) of lakes, ponds, and running waters; only slight use has been made of it in pollution studies, e.g. Caspers & Schultz (1962). The flora is often composed largely of diatoms and can easily be sampled by removing a small amount of sediment, placing this in a petri dish, pipetting off the excess water, and placing cover-glasses on the moist mud surface (Round, 1953). The diatoms are phototactic and quickly rise onto the undersurface of the cover-glass, which can be removed and examined under the microscope. In this way only the living species are recorded; methods involving cleaned preparations frequently give a false impression owing to contamination by dead frustules from other zones. This flora is very sensitive to changes in the habitat; in organically polluted waters there is often a dominance of *Nitzschia* species; in waters polluted by salt wastes, a whole range of halophilic diatoms occur, *e.g., Caloneis amphisbaena, Amphora* spp., *Amphiprora* spp., *Bacillaria paradoxa, Nitzschia obtusa, Cylindrotheca gracilis,* etc. In marine habitats pollution affects this epipelic flora by increasing the flagellate count, especially *Eutreptia* spp., and favouring *Nitzschia* spp. almost to the exclusion of all others. The details of this community in waters of varying degrees of pollution have not been fully worked out, and since it is easy to sample and is almost always present it is worthy of greater study. In the Weser, Hustedt (1957) cites *Melosira varians, Nitzschia communis,* and *Surirella ovata* as abundant saprophytic forms of the littoral zone. These are abundant in many mesosaprobic streams and rivers; *S. ovata* in particular is tolerant of quite high salinities. The epipelic flora is, however, one that may be eliminated by certain types of pollution simply by covering with wastes; e.g. many streams and rivers in industrial regions have a layer of coal dust over the sediment. When conditions are so extreme, however, as to eliminate this flora by physical means, then few if any other algal species will develop.

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* The movement of planktonic organisms does indeed render them less valuable as indicators of pollution, a fact pointed out by Fjerdingstad (1960) leading him to devise a system in which benthic algae are used.

The attached flora has probably received more attention than any other; rarely, however, has the actual flora of rock or stone surfaces, wood piles, or aquatic plants been investigated although numerous workers have pointed out the value that might result from a study of these habitats. This flora like the epipelic would yield valuable indicators of degrees of pollution if a detailed study were made; for example, abundant growths of *Diatoma* are found frequently near outfalls of domestic and farm sewage. This attached flora is the one found growing on glass slides, etc., suspended in the polluted water; perhaps because it is easy to observe, the natural habitats have been neglected. The slides are first colonized by bacteria and then followed by an attached flora often containing a high proportion of diatoms, but almost certainly selective of those that can attach to a smooth surface. The degree of growth and the type of flora on these artificial substrata have been used to indicate the degree of pollution with considerable success, but one disadvantage is that the sampling apparatus must be left in the water long enough for colonization and growth to occur on the slides. Instead of waiting for this, sampling of the natural surfaces and of the sediments can give an answer within 24 hours. The claim that all the species of the water system will sooner or later be found on the slides is undoubtedly true, but equally true is the fact that this method will yield no true picture of the flora of the microhabitats within the water and so there is little opportunity to accumulate the necessary details of the diatom flora of actual microhabitats. A detailed quantitative comparison of the diatom flora of glass slides and the natural attached communities is much to be desired.

The use of diatom indicator species in pollution studies may be extremely unreliable unless other features are also considered. Since (organic) pollution in its milder stages often enhances the growth of species it is necessary to determine the indicator species of this stage from a detailed study of the diatoms of each habitat and to compare the floras of these habitats with similar but unpolluted ones. This use of indicators tends to be very subtle; e.g. the enhanced growth of *Asterionella,* etc., in a Finnish lake quoted above. The more advanced stages of pollution affect the balance of the communities to a greater extent, and finally so unbalance them that only one or a few species remain, which are able to grow to densities unknown in normal balanced systems. Thus these indicators, which they undoubtedly are, can only be used when they are considered in relation to the population as a whole. Much confusion has arisen from listing indicator species that are merely components of a more or less normal flora; e.g. *Nitzschia palea* is abundant in many habitats that would not rank as anything more than mesosaprobic; however, its occurrence in abundance as the sole diatom is an indicator of polysaprobic conditions. In a sense all that is needed for an indication of pollution is the number of species in the habitat, for in all unbalanced ecosystems a single species or a few species remain and frequently grow abundantly. This occurs in natural habitats of extreme type, e.g., highly mineral waters. It is probably true that no diatom species are confined to polluted waters since this is an unnatural state (cf. other groups of organisms); this complicates the use of indicator species. Diatoms can, however, be used as indicator species for certain ionic balances; e.g., increase in salinity is readily detectable, increase in calcium content can also be detected, and also increase in acidic ions. Detailed studies of other species will undoubtedly reveal other useful indicators.

Hustedt's study of the R. Weser showed that the detection of incipient pollution from a study of records is feasible, but this requires long term observations that are rarely available except on important waterways or sources of supply for human consumption. Since diatom cell walls are relatively indestructible, however, they are preserved in sediments of lakes and in the littoral zone of the sea. The sedimentary material is treated in the normal way to obtain slides for diatom analysis. Special sampling techniques are necessary to prevent disturbance of the cores and a fairly thorough knowledge of the tolerance of the species is required before the data can be interpreted. It is possible, however, to detect the time at which pollution started and any acceleration of the process. Normal floras do, however, undergo cyclical changes of varying degree, so the data need to be treated cautiously.

The effect of pollution on the metabolism of diatoms is worthy of serious consideration since it may indirectly supply useful information on the range of their metabolic processes. It may be valuable also to attempt to distinguish between the absence of necessary nutrients or their unavailability in polluted waters and the presence of toxic compounds. The diatoms, unlike almost all other plants, require a source of silica in addition to the usual carbon. This element is usually in adequate supply in natural waters, but anything that prevents leaching of silicates from soils or underwater sediments will prevent diatom growth. Thus in some industrial disposal systems, diatom growth may be completely prevented by blanketing of the source of silica supply. Apart from availability (and rarely will silica be unavailable in polluted natural waters) there is the important aspect of its incorporation into the diatom membrane; in this field there is almost no information regarding mechanism or inhibitors. Geissler (1958), however, showed that the silica wall has a membrane potential and will absorb positively charged particles that reduce the motility of *Nitzschia*, a common genus in polluted waters. It is an interesting fact that in some habitats and often in polluted waters the normally motile diatom species are immobilized. This may of course affect the method of sampling outlined above. The uptake of silica is an aerobic process, dependent upon respiration and almost certainly high energy phosphate. Few diatoms tolerate low oxygen concentrations. Thus, the absence of almost all diatoms in polysaprobic water may be due to the inability to assimilate silica at low oxygen tensions. *Nitzschia* appears to tolerate low oxygen tension and this may be the factor responsible for its abundance in mesosaprobic waters and its penetration into polysaprobic waters.

Although diatoms are photosynthetic organisms it has been known for some time (Lewin, 1935) that they are capable of assimilating organic compounds in the dark. To what extent they do this in natural populations is unknown, although there seems little doubt that it must occur; polluted waters are the most likely place because of the supply of organic substrates. Diatoms may in fact effect some purification by utilization of these organic compounds, as for example *Chlamydomonas* in sewage lagoons (Eppley & Maciasr, 1962). This also means that light is not essential for all diatoms and that in waters of high turbidity due to pollution diatoms need not be lacking. In fact, in turbid littoral marine habitats the rich *Nitzschia* flora on the segments probably lives at very low light intensities; the author has found a very rich diatom flora at 35 m. off the Pacific coast where light intensity is less than 2 percent of surface illumination (Talling, 1960). In unpolluted freshwaters, however, diatoms are not at all abundant below the level to which approximately 5 percent of the light penetrates; this suggests a greater heterotrophic tendency in the marine forms. A study of diatom depth distribution in polluted waters may be revealing. Normal photosynthesis is only likely to be limited by lack of carbon dioxide or of metallic ions associated with the pigments or processes. In addition metallic ions may inhibit this assimilation. Certain algae can be adapted to concentrations of poisonous ions (Cu and Rb ions) and in some instances clones of resistant types arise (Kellner, 1955). To what extent this happens with diatoms is unknown. Lund (1957) showed that the diatom *Asterionella* contained large amounts of nickel (83μg), lead (68μg), titanium (1100 μg), and zinc (1240 μg), whereas a dismid *Staurastrum* contained only 30, 10, 60 and 715 μg. respectively per gram dry weight.

From a pollution aspect these data suggest that certain diatoms may act advantageously in absorbing harmful ions, which would then be released slowly as the diatoms decayed. Marine species also absorb ions in excess of their normal requirements. In effect this is part of the process of self purification in all polluted waters, and thus anything done to increase the algal flora undoubtedly assists self purification. The same principle also operates in the absorption of radioactive wastes. Another point is that some algae, e.g. the *Staurastrum* noted above, obviously cannot absorb large quantities of heavy metals; this may be related to their extensive mucilage sheath, which prevents penetration of the ions. This in itself is important in pollution since these are the very species that may be tolerant at least to mild inorganic pollutions and are hence to be encouraged for their contribution to oxygenation. In view of the tolerance variability of different strains of diatoms to organic factors it would be surprising if no variation of tolerance occurred in their inorganic nutrition. Only isolation and experimental study of species with regard to metallic ions will solve this; if diatoms can be found that tolerate certain metallic ions then these may be valuable in purification of waters polluted by inorganic wastes. Such species are most likely to be found amongst the epipelic and attached diatom flora, e.g. *Nitzschia* or *Cocconeis* species, and not in the plankton where species in general are more sensitive, e.g. the absence of any characteristic diatoms in the sulphite pollution zone in a Finnish lake (Jarnefelt, 1961).

Organic pollution may be more tractable since we now know that some diatoms utilize organic molecules. The range of molecules utilized and the number of species tested experimentally, however, is small. Organic acids are capable of being utilized provided other conditions of the habitat are suitable. The effects of aeration, temperature, pH control, etc., are worthy of further study in the hope that systems can be evolved that will facilitate heterotrophic diatom growth and hence reduce pollution.

The effect of the diatoms on the polluted habitat are at least threefold. By absorbing nutrients they remove considerable quantities of chemicals from the habitat; by their photosynthesis they oxygenate the waters, which is one of the most important contributions to the system; and thirdly they secrete compounds that act as antibiotics and sources of organic matter probably of non-polluting nature. The latter feature has recently been demonstrated with cultures of *Nitzschia palea*, which in the light only produced an extracellular substance that is antibacterial to *E. coli* (Emeis, 1956). Apart from its obvious value in organically polluted waters where the bacterial count is high, it is useful on slow sand filters in waterworks and on sewage beds. Thus Emeis (1956) found that water when passed through an experimental laboratory filter coated with *Nitzschia palea* had its *E. coli* count reduced by 50 percent. Some species of diatoms produce extracellular capsules of carbohydrate nature and all diatoms extrude mucilage to some extent; this diffuses into the medium and may complex with metallic ions.

SUMMARY

A more precise quantitative correlation between the composition of the diatom flora and the habitat conditions is required. A detailed study of the diatoms in the aquatic microhabitats will almost certainly reveal more reliable indicator species for the varying degrees of pollution. The tolerance of diatoms to mixtures of pollutants and the frequent stimulation of diatom growth by mold pollution requires experimental study, which will probably yield important fundamental data on diatom nutrition. Now that it is clear from experimental work that diatoms utilize organic compounds, absorb inorganic ions greatly in excess of their requirements, and produce antibiotics and other extracellular products it will be fruitful to investigate these effects in polluted waters. Since diatoms play an important part in the purification of sewage and of water supplies, it is probable that as more is known about their biology, systems may be designed utilizing diatoms for the breakdown of pollutants.

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THE IMPORTANCE OF EXTRACELLULAR PRODUCTS OF ALGAE IN THE AQUATIC ENVIRONMENT

*G. E. Fogg**

In the words of J. C. Smuts (1931) "There is an overflow of organic wholes beyond their apparent spatial limits." In making this remark Smuts had in mind, not, perhaps, an overflow so excessive as to be described as pollution but the whole complex of interactions between an organism and its environment; it is with one aspect of this subtler kind of overflow as it occurs in algae that this paper is concerned. The mechanical separation of the cells of an alga from the water in which they are growing is so easily accomplished in the laboratory that we overlook the fact that we thereby disrupt an organic system and that the filtrate or supernatant that goes down the drain may be something more than a solution of unused nutrients. Among other things, it may contain metabolites that have been liberated from the cells and which play a vital part in determining the activities of the alga itself or of associated organisms.

Oxygen is, of course, an extracellular product of prime importance, both generally and with respect to pollution, but the possible significance in the aquatic environment of organic substances excreted from algal cells is less obvious. There is, however, abundant evidence that a variety of different organic substances is liberated into the medium from healthy algal cells in laboratory culture. This includes carbohydrates, fatty acids, amino acids, polypeptides, growth substances, vitamins, antibiotics, and enzymes; the total amount of extracellular material in a culture may sometimes be as much as that of the cell material itself. Conditions in laboratory cultures may, of course, be vastly different from those in natural environments but so far there is little evidence to suggest that similar excretion does not occur under natural conditions, and the presence in natural waters of appreciable concentrations of many of the substances concerned has been established (Fogg, 1962). The possibilities of extracellular products of algae having effects of biological importance in aquatic environments are multifarious. In this paper attention is confined to three examples, investigation of which the author has personally conducted.

Firstly, the possibility of extracellular products producing biological effects through chelation of ions is considered. Excretion by algae of substances that appear to be of the nature of polypeptides is evidently common, although particularly evident in the blue-green algae (Fogg, 1962); such substances would be expected to possess chelating properties. By various methods, including electrometric titration, Fogg and Westlake (1955) were able to show that a partially purified preparation of the extracellular polypeptides of *Anabaena cylindrica* forms complexes with various ions including those of copper, zinc, ferric iron and certain organic substances. The value of added chelating agents in algal culture media is well known. Besides maintaining trace elements in available form they may render a medium more favorable for growth by altering the ratios of major ions; e.g., chelating agents improve the growth of *Monodus subterraneus* (an obligately phototrophic coccoid member of the Xanthophyceae) under certain conditions by reducing the effective calcium/magnesium ratio (Miller and Fogg, 1958). Extracellular polypeptides may act in similar ways. The potential biological importance of this for *Anabaena cylindrica* has been demonstrated by showing that complex formation between its extracellular products and cupric ion considerably reduces the toxicity of the latter towards the alga (Fogg and Westlake, 1955). Peptide nitrogen occurs dissolved in unpolluted fresh waters (Hutchinson, 1957) in concentrations that are large relative to those of many biologically important inorganic ions. This peptide nitrogen may originate only partly from living algae but, whatever its origin, it probably exerts appreciable effects on the growth of organisms in the water by forming complexes with other dissolved substances. Possible effects might be to reduce the efficiency of copper sulphate as an algicide, or to permit algal growth in waters polluted with high concentrations of heavy metal ions. Thus high peptide concentrations such as have been reported for Lake Mendota by Domogalla et al. (1925) would probably be sufficient to render non-toxic 0.05 milligram of copper per liter of water, a concentration which under other circumstances would be lethal to most algae.

The second example, the production of extracellular enzymes by algae has scarcely been considered, although the ability of some species to utilize substrates of high molecular weight, such as starch and proteins, suggests that these are sometimes produced. *Monodus subterraneus*, which is able to utilize a variety of organic substances as nitrogen sources (Miller and Fogg, 1958), has been shown by Miller (1959), however, to liberate an extracellular glutaminase. Cell-free filtrates from young cultures of the alga grown with either glutamine or nitrate as the nitrogen source catalyse the hydrolysis of glutamine to glutamic acid and ammonia, and this property is destroyed by boiling. The enzyme is specific, neither iso-glutamine nor asparagine being hydrolyzed by it. *Chlorella vulgaris* and several species of *Xanthophyceae* have been found not to produce a similar enzyme (Miller, unpublished). It is impossible to say at present how widespread such liberation of extra-

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cellular enzymes may be, but it is possible that they may be concerned to an appreciable extent in the breakdown of organic matter in fresh water and thus help to bring about the abatement of pollution. As far as I am aware, no investigations have been made of the enzymic activity of fresh waters filtered to remove all bacteria and other organisms; there are indications, however, that sea-waters may contain dissolved enzymes (Harvey, 1955).

Thirdly, I should like to consider the excretion of glycollic acid by plankton algae, a phenomenon which various pieces of evidence indicate as being of particular importance in the aquatic environment.

It is now well established that algae such as *Chlorella* liberate an appreciable proportion of the carbon, which they fix in short periods of photosynthesis in laboratory experiments in the form of glycollic acid (Tolbert and Zill, 1957; Warburg and Krippahl, 1960; Pritchard, Griffin and Whittingham, in press). In addition glycollic acid has been demonstrated in filtrates from laboratory cultures of *Chlamydomonas* spp. (Allen, 1956; Lewin, 1957) and a planktonic strain of *Chlorella pyrenoidosa* (Nalewajko, Chowdhuri and Fogg, in press). Pritchard et al. (in press) found that glycollic acid is the principal extracellular product of *Chlorella*, except at very alkaline pH values and high concentrations of carbon dioxide; their results suggest that it accumulates when the rate of photosynthesis is carbon dioxide limited. Glycollate can be reabsorbed by *Chlorella* and results of various studies indicate that there is an equilibrium between its intracellular and extracellular concentrations. A particularly interesting finding is that concentrations of glycollic acid of the order of 1 milligram per liter are effective in abolishing the lag phase in the growth of *Chlorella pyrenoidosa* under light-limited conditions. Other organic substances such as glucose, and acetic, pyruvic, and malic acids do not do this although they may increase the total amount of growth in the culture. This suggests that following inoculation of this *Chlorella* into fresh medium, glycollate is the major product of photosynthesis and only when an equilibrium concentration of this substance has been built up in the medium does a sufficient proportion of the output of the photosynthetic fixation become available to maintain exponential growth (Nalewajko et al., in press).

The available evidence suggests that similar excretion by phytoplankton occurs under natural conditions. We (Fogg and Nalewajko, unpublished) have now carried out a great many measurements of photosynthesis over periods of 3 to 24 hours, *in situ* in lakes, by the radiocarbon method of Steemann Nielsen (1952), in which, in addition to fixation of radiocarbon in particulate matter, fixation of radiocarbon in dissolved organic matter has also been determined. These measurements have shown that fixation in extracellular organic matter always occurs, and amounts to 5 to 90 percent of the total fixation. This extracellular fixation is proportional to the amount of photosynthesis that has taken place, negligible amounts of C^{14} being fixed in the filtrates from samples kept in dark bottles. The principal extracellular product produced by phytoplankton in short-term experiments seems to be glycollate, just as in the case of *Chlorella*. The radiocarbon fixed in organic substances can be recovered completely from the filtrates from natural phytoplankton samples by distillation *in vacuo* after acidification. Extracellular fixation is thus largely in volatile substances and glycollic acid can be detected in the distillates. If the extracellular substances in which radiocarbon is fixed were derived from broken cells they would be largely non-volatile. These results support the idea that glycollic acid is qualitatively the most important extracellular product of phytoplankton under the conditions of these experiments, but unequivocal proof of this has yet to be obtained. Attempts to demonstrate labelling in extracellular glycollate by C^{14} supplied as bicarbonate with radio-autography of chromatograms have so far been unsuccessful.

Glycollic acid is, however, normally present in lake waters. We have demonstrated its presence, qualitatively, in water from Windermere by paper chromatography. Direct colorimetric determination of water samples is unreliable; it has been found best to extract the acid into ether by a method similar to that used by Koyama and Thompson (1959) for seawater, after which it may be determined by the colorimetric method of Calkins (1943) with 2:7 dihydroxynaphthalene. Some results obtained by this method are as follows:—

		Glycollic acid	
Windermere, North Basin	April 1961	0.29	mg./liter
Windermere, North Basin	October 1961	0.045	" "
Ennerdale Water	April 1961	0.12	" "
Bassenthwaite	April 1961	0.13	" "
Blelham Tarn	July 1961	0.12	" "

Kamata (unpublished) who estimated the amounts of various organic acids in the waters of Lake Kizaki-ko, Japan, by titration after separation by chromatography on silica gel, also found glycollic acid but in smaller concentrations than those presented above.

As far as it goes, this evidence strongly suggests that plankton algae under natural conditions normally liberate glycollic acid from their cells during photosynthesis. The extent of this loss cannot be judged from the results of the radiocarbon determinations reported above, since, if glycollic acid is present in the lake water, there may be absorption simultaneous with the escape of labelled molecules from the cells. Thus, with very low concentrations of glycollic acid in the surrounding water, the radioactivity of organic substances in the filtrate would be expected to be a good measure of the loss of material from the cells, whereas with higher concentrations it might only denote an exchange with no net loss from the cells. The former state of affairs might be expected to obtain at the end of the winter and, if the supposition is correct that growth cannot begin until a certain limiting concentration of glycollic acid has been established in the medium, this may explain the suddenness with which the spring outburst of plankton growth begins. Only when conditions are favorable for photosynthesis and when turbulence has diminished sufficiently for concentration gradients to be set up around the cells can equilibrium be established and the products of photosynthesis become available for the building of cell material. It might be possible to prevent plankton growth altogether if some sufficiently effective means of removing glycollic acid from the water could be found. It would be expected that the position of equilibrium would vary according to conditions so that there may be net absorption of glycollic acid at times. Thus, the finding of Pritchard et al. (in press) that glycollate accumulation in the medium takes place when photosynthesis is carbon dioxide limited suggests that reabsorption of this substance occurs when photosynthesis is light limited and thus that growth may be maintained under these conditions at the expense of dissolved glycollic acid. There is, in fact, some evidence that plankton algae can assimilate dissolved organic substances (Fogg and Belcher, 1961; Rodhe, in press) and grow in darkness (Rodhe, 1955). Other organisms besides algae will, of course, utilize dissolved glycollic acid. Bacteria, such as *Pseudomonas ovalis* and a mutant *Psuedomonas* B2 aba, which will grow on glycollate as sole carbon source, have been investigated by Kornberg and Gotto (1961). Lewin (unpublished) has isolated six strains of fresh-water bacteria able to grow on glycollate as sole carbon source. We have found one of these, provisionally called Gly-1, to be capable of appreciable growth in a medium containing 10 milligrams glycollic acid per liter, and presumably slow growth might be possible at concentrations as low as those found naturally in lakes. Using C^{14}-labelled glycollate we have demonstrated appreciable uptake by *Daphnia* from concentrations of the same order as those found in natural waters, but it is clear that these larger organisms can only obtain a minute fraction of their total carbon requirements from this source. Parsons and Strickland (1962) have suggested that the amount of heterotrophic carbon assimilation by microorganisms in the sea may be a significant fraction of the photosynthetic production, when considering the whole water-column in a deep ocean. Similarly, considering a fresh-water habitat as a whole it may be that a considerable proportion of the primary production of cell material is heterotrophic, taking place at the expense of glycollate excreted by the phytoplankton during photosynthesis.

Our knowledge of the extracellular products of algae is still scanty; in particular, we are ignorant of what may be the most important group from the point of view of pollution control - the antibiotics. Pathogenic and coliform bacteria die out rather quickly in sewage oxidation ponds in which there is abundant algal growth; this could perhaps be the consequence of the production of antibacterial substances. There is evidence that some algal species produce such agents in culture (see Fogg 1962) but it is not yet known how widespread such production is, nor is there much information about the chemical nature of the substances. A great deal more investigation is required. It is hoped that the discussion in this paper will have shown that the extracellular substances of algae may be of considerable ecological importance in aquatic habitats and that account must be taken of them when considering problems of pollution.

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TOXIC WATERBLOOMS OF BLUE-GREEN ALGAE

*Paul R. Gorham**

INTRODUCTION

My part in this symposium is to consider the origin and development of toxic waterblooms of blue-green algae and their effects upon water quality. I shall talk, therefore, about the special case of those waterblooms that cause death of livestock, pets, waterfowl, and other animals. By "waterblooms" I mean the heavy concentrations of floating algae that accumulate (usually in warm weather) in lakes, reservoirs, or ponds that have been naturally or artificially fertilized with growth-promoting minerals and organic substances. I use the term "concentrated waterbloom" to describe the thick, paint-like mess that results when winds blow these algae into shallow, inshore waters.

Most of the well-known cases of poisioning by blue-green algae have involved livestock and pets, but cases of algal poisoning of wild animals and birds have been described. In this paper I shall discuss in some detail what is known about livestock poisoning, and review briefly what is known about the toxicity of blue-green algae towards other animals and man.

Poisonings by blue-green algae have been reported from such varied parts of the world as Australia, Argentina, Bermuda, Brazil, Canada, Israel, U.S.A., Union of South Africa, and U.S.S.R.*(5, 12, 16, 22, 33, 45, 50, 56)*. Fitch *et al.(10)* have described five outbreaks of algae poisoning investigated by the Division of Veterinary Medicine at the University of Minnesota between 1918 and 1934. In one case the death of 1 sheep, 17 hogs, and about 50 chickens occurred a short time after these animals had drunk lake water containing *Coelosphaerium Kützingianum* and *Anabaena flos-aquae*. In another case, nine cattle died in about one hour and a half after drinking from a lake containing a concentrated waterbloom of blue-green algae. In the same pasture there were horses, sheep, hogs and other cattle that did not seem to be affected, however. Olson*(33)* has reported that a concentrated waterbloom in Fox Lake, Minnesota, killed a large number of hogs, ducks, chickens, and other animals after they had consumed small quantities of it. When the wind changed direction the algae accumulated on the opposite shore and there killed 79 hogs, 2 horses, and an uncounted number of ducks, cats, dogs, wild animals and birds. A 0.1 milliliter sample, consisting predominantly of *Anabaena Lemmermannii (An. flos-aquae)* killed mice within 20 minutes, but 2.0 milliliter from a sample collected from the same spot 9 days later killed animals only after 5 hours. In 1949, Stewart, Barnum, and Henderson *(49)* investigated an outbreak of algal poisioning in Sturgeon Lake, Ontario. Six gallons of concentrated waterbloom, consisting predominantly of species of *Microcystis*, *Anabaena*, *Nostoc*, and *Lyngbya* was administered by stomach tube to an 1100-lb cow and caused her to die within 25 min. A 0.1 milliliter dose given intraperitoneally was sufficient to kill mice. A 2.0 milliliter dose was required to kill mice with another sample from the same source collected 10 days later. The latter sample consisted chiefly of *Anabaena* with some *Microcystis*. A third collection, made 2 months later when algal growth again appeared heavy, was non-toxic even when concentrated 10 times.

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These cases, and others that might be cited, illustrate the kind of evidence for the conclusion that waterblooms are in some way and under some conditions responsible for the sudden deaths of livestock and other animals. These cases also illustrate how variable the apparent toxicity of waterblooms can be. Species composition undoubtedly contributes to this variation, but changes in toxicity have been observed even when there has been no apparent change in the species composition of the bloom *(34)*. Waterblooms consisting predominantly of the following six species* have been implicated in algal poisonings *(10, 16, 22, 37)*: *Microcystis aeruginosa* Kütz.em. Elenkin(including *M. flos-aquae* and *M. toxica*), *Anabaena flos-aquae* (Lyngb.) de Breb. (including *An. Lemmermannii*), *Aphanizomenon flos-aquae* (L.) Ralfs, *Gloeotrichia echinulata* (J.E. Smith) P. Richter, *Coelosphaerium Kutzingianum Nag.*, and *Nodularia spumigena* Mert. What causes waterblooms dominated by one of these species to be toxic at one time and not another is a problem that has long puzzled investigators. Various workers have shown that bacterial infections or recognized toxins, including botulinus toxin, are not involved *(10, 33, 58)*. Louw *(25)* has suggested that *Microcystis* toxin is an alkaloid.

LABORATORY STUDY

About 8 years ago a laboratory study of this problem was initiated in the Division of Applied Biology of the National Research Council of Canada at Ottawa. Our long-range objectives were to answer the following questions:

1. How many toxins are involved?
2. Do the algae produce all of the toxins or do the associated bacteria also produce some?
3. What is the chemical nature of each toxin?

The plan of study we adopted was as follows:

1. Locate waterblooms of the suspected species and test them for toxicity.
2. Isolate and culture strains of suspect species from toxic and non-toxic waterblooms.
3. Grow each strain in sufficient quantity to test its toxicity on mice.
4. Establish the algal or bacterial origin of each toxin.
5. Mass culture representative strains and confirm their toxicity on large animals.
6. Isolate and determine the chemical structure of each toxin.

We concentrated most of our attention on waterblooms of *Microcystis aeruginosa* so a great deal of what I have to say about waterbloom toxicity is based on conclusions drawn from these studies *(3, 12, 14, 21, 28, 29, 46, 59)*. We recently carried out some studies on toxic waterblooms of *Aphanizomenon flos-aquae* and *Anabaena flos-aquae* and I shall deal with these, as far as they have gone, after discussing toxic *Microcystis*.

MICROCYSTIS

The very first unialgal isolate of *Microcystis aeruginosa* we managed to grow proved to be toxic to mice when administered intraperitoneally or orally. It produces an endotoxin, which we call the fast-death factor (FDF)*(21)* because a minimal lethal dose kills mice in 30 to 60 min. It produces symptoms of pallor followed by violent convulsions and prostration. Upon autopsy, the liver has a mottled appearance caused by cellular breakdown. The bacteria associated with the alga also produce a toxin (or toxins), which we called the slow-death factor (SDF). This produces symptoms of piloerection, dyspnea, and lethargy and a minimal lethal dose causes death within 4 to 48 hr. The presence of SDF is frequently obscured by the presence of FDF. Attempts to obtain a bacteria-free culture of toxic *M. aeruginosa* NRC-1 have been unsuccessful so far, but the results of toxicity tests on algal and bacterial fractions and other evidence are consistent with the conclusion that the origin of FDF is the alga and the origin of SDF is the bacteria *(12, 46)*. That FDF is an endotoxin is shown by the fact that fresh suspensions of cells are non-toxic. They become toxic, however, if they are frozen and thawed, sonic-disintegrated, or decomposed by semi-anaerobic incubation *(21, 58)*. In nature, FDF must be released by decomposition. We mass-cultured *M. aeruginosa* NRC-1 *(31, 12)* in a medium called No. 11, developed for maximum yield *(21, 50)*. We produced a continuous "laboratory bloom" on a scale sufficient to yield 1 to 2 kilograms of freeze-dried cells per month.

In collaboration with the Animal Diseases Research Institute, Canada Department of Agriculture, Hull, Quebec, toxicity tests on large animals have been carried out. Freeze-dried *Microcystis* cells containing FDF killed a variety of animals when administered both orally and intraperitioneally *(12, 14)*. Domestic ducks were resistant, however, so it is unlikely that FDF caused waterfowl sickness. The symptoms and autopsy findings agreed quite well with those described for toxic *Microcystis* waterblooms *(1, 27, 48, 50)*. A sample was sent to Prof. Douw G. Steyn in Pretoria, South Africa. He kindly tested it on laboratory animals and reported that the effects were indistinguishable from those he had observed in 1943-45 during his investigation of *Microcystis* poisonings in the Transvaal. On this evidence, we have concluded that *Microcystis* FDF is one of the important algal toxins.

With large quantities of toxic *Microcystis* cells available, it was possible to tackle the problem of isolating and identifying FDF. This proved to be a difficult task, since, at the outset, we had no diagnostic chemical reactions. This meant that fractionation and purification had to be guided by bioassay with mice. Finally we were able to show that FDF was not an alkaloid, as Louw *(25)* has suggested, but one of five closely related polypeptides *(3)*. Its molecular weight is low, since it dialyzes slowly through Visking casing, and it appears to be cyclic. Upon hydrolysis it yields seven amino acids in the following molar proportions:

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* Nomenclature according to Prescott, G.W. Algae of the Western Great Lakes Area. Rev. Ed. Wm. C. Brown Co., Publishers, Dubuque, Iowa, 1961.

aspartic	1
glutamic	2
D-serine	1
valine	1
ornithine	1
alanine	2
leucine	2
	10

The MLD (IP) for mice of pure FDF is 0.47 milligram per kilogram body weight, so it is only a moderately toxic substance. FDF and the antibiotics bacitricin and gramicidin S have a number of properties in common. All three are cyclic polypeptides of low molecular weight. FDF, however, does not inhibit the growth of such bacteria as *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, or *Pseudomonas hydrophila (3)*, nor does it possess antigenic activity *(45)*.

Our work *(12, 14, 46)* and that of Thomson et al. *(51, 52)* of the Defence Research Board of Canada indicate there is more than one SDF produced by the different bacteria found in association with *Microcystis* and other blue-green algae. Thompson et al. found in a survey of 25 algal collections that Gram-negative rods were most common. Twenty-two out of twenty-six isolates of such Gram-negative bacteria produced a heat-stable acid-stable, acetone-precipitable neurotoxin. The chemical structure of this and other slow-death factors is still unknown, however. The presence of large numbers of bacteria in a waterbloom is an additional complication that must be considered in ascertaining the cause of any toxicity.

We tested a total of 10 *Microcystis* waterblooms for toxicity and found that 7 contained FDF while the remainder were either non-toxic or contained SDF. Nineteen colonial isolates of *M. aeruginosa* have been grown and tested. Only eight produce FDF while the rest are non-toxic or produce SDF. We attempted, without success, to induce the production of FDF in a non-toxic strain of *M. aeruginosa* by isolating and transferring the bacteria from *M. aeruginosa* NRC-1 *(46)*. Hence, it appears that FDF production is controlled genetically as well as physiologically.

APHANIZOMENON

There are at least five case histories on record of poisonings attributed to waterblooms consisting mainly of *Aphanizomenon flos-aquae (22, 31, 32, 3[illegible], 36, 37)*. Wheeler et al. *(58)* tested almost pure *Aph. flos-aquae* from Minnesota on mice without effect. In 1955, we collected a bloom of almost pure *Aph. flos-aquae* from the Rideau Canal, Ottawa, Ontario, freeze-dried it, and tested its toxicity on mice. It produced no fast deaths, only slow deaths at high dosage. In 1960, through the cooperation of Professor H.K. Phinney and Mr. C.A. Peek of the Department of Biology, University of Oregon, we obtained three fresh samples of bloom, consisting of *Aph. flos-aquae* and *M. aeruginosa* in about equal proportions, from Klamath Lake, Oregon. These samples were freeze-dried for testing. We also obtained a dried sample of toxic bloom of similar composition collected by Phinney and Peek from Klamath Lake in 1957 *(35)*. As judged by the symptoms and survival times when administered intraperitoneally to mice, all four samples contained only *Microcystis* FDF. One strain of *Aphanizomenon* isolated from the Rideau Canal bloom and four strains isolated from a bloom collected in 1960 from Burton Lake, Saskatchewan *(30)* are non-toxic, or produced only small amounts of SDF. More work on the toxicity of *Aphanizomenon* is needed, but from the evidence available so far it appears that this species either produces a toxin that is indistinguishable from *Microcystis* FDF or is non-toxic.

ANABAENA

There are at least three case histories on record of rapid deaths of livestock, poultry, waterfowl, and other animals that are attributed to the consumption of waterblooms composed mainly of *Anabaena flos-aquae (An. Lemmermannii) (7, 9, 33, 42)*. Survival times as short as 2 min have been reported. This fact, together with differences in water-fowl susceptibility *(12)* (Olson, private communication) suggests that a toxin that is different from FDF is probably involved. We tested the toxicity of seven waterblooms consisting mainly of *An, flos-aquae*. Two freeze-dried samples, collected from the same location from Little Rideau Lake, Ontario, in June and October, 1955, contained only SDF. Five samples collected from Burton Lake, Saskatchewan in 1960 and 1961, however, killed mice within 1 to 10 min after intraperitoneal or oral administration of a minimal lethal dose *(13)*. These very fast deaths were preceded by paralysis, tremors, and mild convulsions. Upon autopsy the liver appeared normal. Between 50 and 95 percent of this very-fast-death factor (VFDF) was found in the water. Freeze-dried samples of Burton Lake bloom were sent to Professor L.D. Jones, D.V.M., South Dakota State College, and Professor T.A. Olson, University of Minnesota, for comparative tests. Both reported that symptoms and survival times when tested on laboratory animals were indistinguishable from those observed with *An. flos-aquae* during the outbreak of algal poisonings at Storm Lake, Iowa, in 1952 *(9, 42)* and with toxic blooms from Minnesota *(33, 34)*, respectively.

We succeeded in growing 14 strains of *An. flos-aquae* isolated from two of the waterblooms collected in Burton Lake in 1960 and 1961. Cells or culture filtrates from eight strains contain typical VFDF while those from six strains do not.

Anabaena VFDF acts much more rapidly than *Microcystis* FDF. This suggests that it is a smaller molecule. *Anabaena* VFDF is soluble in water or ethanol, insoluble in acetone, ether or chloroform like *Microcystis* FDF *(3)*, but VFDF kills waterfowl *(33, 42)* whereas FDF does not. Olson *(33)* isolated a quantity of *Anabaena* VFDF from a waterbloom and separated it into several toxic fractions, one of which has a strong absorption between 210 and 290 millimicrons. The chemical structure of VFDF and its relation to FDF await further study. This should be possible now that we have toxic strains to produce it.

OTHER SUSPECTED SPECIES

We have not located any waterblooms dominated by *Gloeotrichia echinulata*, *Coelosphaerium Kützingianum*, or *Nodularia spumigena*. However, we have grown and tested the toxicity of one strain each of *G. echinulata* and *C. Kützingianum* (from the Cambridge Culture Collection) and *Nodularia sphaerocarpa* Born. et Flah. (from the Indiana Culture Collection). All three are non-toxic. More blooms and strains of these suspect species need to be tested before we can decide whether they, too, produce toxic strains.

TOXICITY OF WATERBLOOMS

The work described so far provides at least partial answers to the questions concerning the toxicity of waterblooms of blue-green algae towards livestock and other mammals. We can conclude that: (1) More than one toxin may be involved; (2) the principal toxins appear to be algal in orgin; (3) bacteria may also produce toxins; (4) one of the major algal toxins is a polypeptide, while another, not yet identified, appears to be different; (5) depending on the species involved, algal toxins are either excreted or released into the surrounding water by decomposition; and (6) the variable toxicity of waterblooms arises from a complex of different factors. These factors are too numerous to discuss in detail, but they include differences in algal and bacterial strains, species, culture conditions, age, accumulation, secretion, and decomposition, and also differences in toxin destruction or inactivation, dosage, and animal susceptibility. It is a curious fact, mentioned by Singh*(47)*, that many experiments in India with several types of blooms and different animals (guinea-pigs, rabbits, squirrels, etc.) have given no indications of toxicity even though the same species are involved as those in other countries where toxicity is encountered. Whether this reflects strain differences, physiological differences, or both, has not been determined.

AQUATIC ANIMALS

A number of reports indicate that waterblooms of blue-green algae are sometimes toxic to other animals besides mammals and birds. The toxic factors involved are not necessarily the same in every case, of course. Prescott *(36)* and Mackenthun et al. *(26)* have described experimental fish kills with survival times that varied from 1 or 2 hours up to 34 hours, which were caused by decomposition of concentrated waterblooms of *Aphanizomenon*. At no time did the dissolved oxygen content drop sufficiently low to cause death. Prescott analyzed the water and found that toxic concentrations of hydroxylamine were present. Hydrogen sulphide was also present at 8.5 ppm; he suggested that it might be an additional toxic agent. Lefèvre et al. *(24)* described a fish kill in Morocco involving a waterbloom of *Microcystis*. Here, too, carp died despite the presence of adequate amounts of dissolved oxygen. Shelubsky*(45)* showed that carp injected intraperitoneally with toxic *Microcystis* cells (containing FDF) died within 1 to 4 days. Astakhova et al.*(2)* concluded from experiments that both fresh and decomposed samples of a waterbloom consisting of *M. aeruginosa*, *Aph. flos-aquae* and *An. flos-aquae* were highly toxic to carp and that such waterblooms were responsible for the widespread disease of carp that has been observed in the Volga delta since 1956.

Shelubsky *(45)* reported that suspensions of *Microcystis* (containing FDF) injected intraperitoneally and subcutaneously were toxic to frogs. Prescott *(37)* observed periodic deaths of large numbers of mudpuppies *(Necturus)* that he correlated with blooms of *Aph. flos-aquae*. Gurevich*(17)* reported that *Oscillatoria* stunts the development of frog embryos and that they eventually die. Braginskii*(4)* tested the toxicity of decomposing waterblooms towards *Daphnia*, *Cyclops*, and other zooplankton. He reports that *Microcystis* was exceptionally toxic and *Aphanizomenon* was slightly toxic, while blooms of two species of *Anabaena*, *Gloeotrichia natans*, and the green alga, *Volvox*, were harmless. Dillenberg and Dehnel*(8)* recently reported that 0.5 milliliter of a heavy bloom consisting of *Anabaena* and *Aphanizomenon* killed mice within 8 hours and immobilized *Daphnia* within 30 minutes.

Only a few of the toxic effects on animals other than mammals and birds have been adequately corroborated or systematically investigated. Most await detailed study to find out whether the algae are really to blame, either directly or indirectly, for the toxicities.

MAN

The subject of algae and medicine has been reviewed by Schwimmer and Schwimmer*(43)*. Heavy concentration of blue-green algae in public water supplies with their resulting organic load has been cited as a possible factor in bringing about the epidemic of human gastroenteritis that occurred in the drainage basins of the Ohio and Potomac Rivers in 1930-31, *(53, 54, 55)*. Dillenberg and Dehnel *(8)* and Senior *(44)* report three cases of human nausea and gastroenteritis following ingestion of blue-green algae in Saskatchewan in 1959. These were investigated by the provincial health service. No *Salmonella* or *Entamoeba* were found in the stools but many cells of *Microcystis* and *Anabaena* were found, which strongly suggested that the ingestion of waterblooms had caused the ailments. Cutaneous sensitization and other allergic responses to blue-green algae have been reported by Cohen and Rief *(6)* and Heise *(19, 20)*.

DEVELOPMENT OF TOXIC WATERBLOOMS

Let us turn, now, from considering the different ways in which waterblooms of blue-green algae can be toxic to a consideration of what little we know about environmental requirements for the development of toxic waterblooms. This problem really has two aspects: (1) the factors influencing the production of a toxic situation at any one time; and (2) the factors that determine whether an animal will consume a lethal dose at the same time.

Little is definitely known about what causes concentrated waterblooms of blue-green algae to develop except that they occur in eutrophic waters during warm, sunny weather. Even less is known about what causes particular (toxic) strains of particular species to become dominant. We studied the growth requirements of one toxic strain of *M. aeruginosa* (NRC-1) and one toxic strain of *An. flos-aquae* (NRC-44) in considerable detail in the laboratory *(13, 21, 59)*. Both species have fairly critical requirements for certain minerals. The requirements for light, temperature, pH, carbon dioxide, and chelators appear to be somewhat less exacting. When the optimum laboratory conditions are compared with the conditions observed with blooms of these species in nature there is very little or no correspondence to be found. This suggests that the conditions that determine the later stages of bloom development may exert their effects at earlier stages, thereby complicating their detection and interpretation.

The effects on waterbloom development of predators (fish, zooplankton), bacteria, and other algae, and such extracellular products as organic acids, amino acids, amides, polysaccharides, polypeptides, enzymes, vitamins, growth promoters and growth inhibitors *(11, 15, 18, 23, 24, 37, 38, 39, 40, 41, 51, 54)* have either not been studied, or their study has barely begun. It is quite evident, however, that a great many variables can affect the development of both toxic and non-toxic waterblooms of blue-green algae.

For poisonings to occur, at least two other conditions must be met besides the development of a waterbloom dominated by toxic strains of algae and/or bacteria: (1) The bloom must accumulate in shallow water so that the concentration of toxins that are liberated will not be reduced too rapidly by dilution, circulation, or adsorption *(3, 45, 58)*; (2) susceptible animals must be present at the right time and consume sufficient quantities of the toxins for the latter to be lethal despite adsorptive detoxication in the stomach and gut.

CONCLUSIONS

Toxic waterblooms of blue-green algae are detrimental to water quality because of the hazard they present not only to livestock, wildlife and pets, but also to fish and other animal life. They are a potential hazard to public health, too, since they may cause gastritis and allergies. They are a nuisance to recreation because of the unpleasant appearance and offensive odors associated with their decomposition. It is apparent that a great deal more study of all types of waterblooms is needed to determine more precisely the factors that are necessary for their development and the effects they have on water quality. These studies should be paralleled and supported by physiological and biochemical studies in the laboratory of bloom-producing algae and associated bacteria. Such studies may never provide entirely satisfactory answers to every question we may ask about such complicated ecological phenomena, but they should provide a better understanding than we have at the present time.

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DISCUSSION

Emphasis was made that in evaluating the effects of pollution upon the biota of a stream, the mere identification of the diatom species present is not sufficient, but quantitative data must be obtained. Also, it was pointed out that it is not adequate to give a range of pollutional tolerance between which a diatom is found. For example, a species of diatoms may be absent where there is low dissolved oxygen. However, the low dissolved oxygen may, in turn, be due to the presence of such things as sulfides or ammonia, and these compounds may, in fact, be responsible for the absence of that particular diatom species and not the low dissolved oxygen.

One discusser expressed the opinion that there should be two expressions for describing an ecological situation; the ecobalance and ecoexistence and that ecoexistence could not be properly described by making physiological observations alone.

A great deal of the discussion centered about the methods of diatom sampling in a river or a stream in order to obtain a representative flora. Dr. Round suggested that, insofar as determining the diatom population as related to the degree of pollution is concerned, an examination of the bottom sediment would perhaps be a better method. In his studies he had found little or no comparison between the diatom flora of the sediment and that found on glass slides suspended in the water. Also, since he assumed that a stream contained no true planktonic forms, the flora of the sediment was a more reliable index.

Dr. Patrick suggested that perhaps Dr. Round had not used the glass slides in the same manner as she does, and, therefore, had obtained different results. She said that when a capable diatomist was in the field and collected and identified all the diatom habitats of a particular area where glass slides had also been suspended for two weeks, 70 to 80 percent of the species found on the slides were found in the specimens collected by the diatomist when 8,000 specimens were examined. Of the 20 to 30 percent of those not found by the diatomist, over 90 percent were represented by only six specimens. She also pointed out that her pollutional studies are aimed at finding changes in the structure of the community of diatoms and in order to do this samples must be compared from areas above and below the pollution. Since diatoms are not randomly distributed, this is difficult to do without introducing an artificial substrate. Also, when a glass slide was suspended vertically, a majority of the species present was obtained. However, when suspended horizontally, a great deal of mud adhered to the slide. Dr. Round pointed out that he would expect less of the bottom forms to be present when a slide was vertically suspended.

Dr. Williams pointed out that because water plant operators are interested in knowing what organisms are in the water and the dispersed organisms represent the greatest volume of water, the National Water Quality Network Program (USPHS), is more interested in the diatoms that are planktonic. Also, because of the magnitude of the sampling program, they must limit their microscopic observations to the dominant forms, and it is his feeling that this type of analysis gives results that are indicative of the condition of the stream when samples are taken at the proper time.

Dr. Round agreed that from a waterworks point of view, one needed to know about the so-called planktonic forms. However, in dealing with pollution these are of little value because pollutants are not ordinarily discharged into the middle of a body of water and, as previously pointed out, there is really no true plankton in a stream.

Dr. Patrick agreed with Dr. Round's proposal for the examination of sediments if one is desirous of finding out where the diatoms grow. In a pollution study, however, the evaluation must be carried out as quickly as possible. The study should not be limited to the dominant species because there is a great difference between the dominant species alone and the dominant species plus others.

Other workers confirmed Dr. Fogg's observations regarding the amounts of radioactivity excreted by the algae after they were allowed to assimilate radioactive carbon. It was noted also that extracellular excretion was dependent upon such things as photosynthetic rates and time.

The production of extracellular algal enzymes was also noted. Phosphatase, saccharase (sucrase), and amylase have been found in natural habitats, and there was a correlation between their presence and the annual plankton cycle. It was also pointed out that in the natural habitat *Scenedesmus* takes up substances just before the time of cell division, and, correspondingly, cell division is dependent upon light.

In work concerning the origin of nitrogen in lake water, there is evidence that a great deal of the nitrogen is derived from extracellular products, and the concentration of nitrogen in the water is related to the detention time. Although the nitrogen could result from decaying cells, the evidence indicates that it is liberated also from living cells.

The question was raised as to whether the pH rise observed at the occurrence of a bloom of blue-green algae was the result of the bloom or whether the pH was relatively high in the beginning. It was suggested that the pH was probably high in the beginning. The removal of carbon dioxide, however, by photosynthetic activity and the release of amines by the algae would certainly cause the pH to rise as growth proceeds.

Another worker described the appearance of a bloom of *Oscillatoria rubescens*, after which a fish kill occurred. Although the death of the fish was thought to be the result of oxygen depletion, this observation required a study of the possible toxicity of this species of algae. Apparently there are no reports of *Oscillatoria* being toxic to fish. There are reports of it having influenced the breeding of fishes in Scotland, however.

While there was no information offered during the discussion relating to the toxicity of blue-green algae to insects, it was stated that there was evidence of *Anabaena* controlling the population of mosquito larvae in the rice fields of California.

Cases of human toxicity of blue-green algae were discussed where children and adults, after swimming in water containing the algal bloom, developed high fever and diarrhea. Suspecting malaria, examination of the stool revealed the presence of blue-green algae.

ENVIRONMENTAL REQUIREMENTS OF FRESH-WATER INVERTEBRATES

L. A. Chambers, Chairman*

BACTERIA-PROTOZOA AS TOXICOLOGICAL INDICATORS IN PURIFYING WATER

S. H. Hutner, Herman Baker, S. Aaronson and A. C. Zahalsky†

There is a cynical adage that all travellers become entomologists. But, now with DDT and detergents, travellers and stay-at-homes alike are becoming toxicologists. We have an immediate interest in pollution problems: Our laboratory receives, like the East River and adjoining United Nations buildings, a generous sootfall from a nearby power plant. Also, we have seen a superb fishing ground, Jamaica Bay in New York City, become a sewer. (Jamaica Bay is, however, being restored to its pristine cleanliness--but not the U.N. area.) We take our theme nevertheless not from aesthetics but from statements by Berger (1961): (1) It is an expensive, time-consuming project ". . . to predict with confidence a new waste's probable impact on certain important downstream water uses." And (2) "The toxicological phase of the study is perhaps its most perplexing aspect. The specialized services and cost necessary for determining the effect of repeated exposure to low concentrations of the waste for long periods of time would inevitably place this job out of reach of most public agencies. Equally discouraging, perhaps, is the probability that the toxicological study may take as long as two years."

As described here, advances in microbiology offer hopes of lightening this burden. The first question is: What kind of microcosm will serve for toxicological surveys, especially for predicting the poisoning of biological means of waste disposal? The second is: Can the protozoa of this microcosm predict toxicity to higher animals?

The food-chain pyramids of sewage plants and polluted waters have been adequately described (Hynes, 1960; Hawkes, 1960). A problem treated here is how to scale those microcosms to experimentally manipulable microcosms yielding dependable predictions for the behavior of sewage-plant microcosms.

THE WINOGRADSKY COLUMN AS SOURCE OF INOCULA FOR MINERALIZATIONS AND AS TOXICOLOGICAL INDICATOR SYSTEM

Total toxicity depends on intrinsic toxicity-persistence relationship. Techniques for testing the degradability of organic compounds--and so their persistence in soil and water--are still haphazard. The enrichment culture technique, in which one seeks microbes that use the compound in question as sole substrate--hence degrades it and even "mineralizes" it--was developed by the Dutch school of microbiologists. Enrichment cultures are used routinely by biochemists wishing to work out the microbial catabolic metabolic pathway for a compound of biochemical interest. Since the compounds dealt with by biochemists are of biological origin, microbial degradability can be assumed. Still, finding a microbe to degrade a rare biochemical is not always easy: Dubos, in a classical hunt for a microbe able to live off the capsules of pneumococci, found the bacterium only after a long search which ended in a cranberry bog. Such difficulty in finding microbes that degrade rare biochemicals implies an even greater difficulty in finding microbes that degrade many products of the synthetic organic chemicals industry, since such compounds may embody biochemically rare or biochemically nonexistent linkages. Intimations of the importance of inoculum abound in the literature, e.g., Ross and Sheppard (1956) could not at first obtain phenol oxidizers from ordinary inocula (presumably soil and water), but manure and a trickling filter from a chemical plant proved abundant sources of active bacteria. One wonders how extensive a study underlies the statement quoted by Alexander (1961) that "soils treated with 2,4,5-T (trichlorophenoxyacetic acid) still retain the pesticide long after all vestiges of toxicity due to equivalent quantities of 2,4-D have disappeared."

What then is a reasonable inoculum for testing a compound's susceptibility to microbial attack? The size range is wide: from the traditional crumb or gram of soil or mud to the scow-load of activated sludge contributed by New York City to inaugurate the Yonkers sewage-disposal plant. We suggest that to strike a practical mean in getting a profile of soil, mud, or sludge to be used as inoculum the uses of the Winogradsky column should be explored. Directions for Winogradsky column and bacteriological enrichments have been detailed (Hutner, 1962) and so only an outline is given here. The column is prepared by putting a paste of shredded paper, $CaCO_3$, and $CaSO_4$ at the bottom of a hydrometer jar, filling the jar with mud smelling of H_2S, covering with a shallow layer of water, and illuminating from the side with an incandescent lamp. In 2 or 3 weeks sharp zones appear: a green-and-black anaerobic zone at the bottom (a mixture of green photosynthetic bacteria along with SO_4-reducers, methane producers, and the like); over that a red zone (predominantly photosynthetic bacteria); above this garnet or magenta spots or zone (predominantly non-sulfur photosynthetic bacteria); above this a layer rich in blue-green algae (the transition to the aerobic zones); above this aerobic bacteria along with green algae, diatoms, other algae, and an assortment of protozoa. This makes an excellent simple classroom experiment to demonstrate the different kinds of photosynthetic organisms, especially the bacterial forms that are important in photosynthesis research and that ordinarily escape notice yet are ubiquitous in wet soils and natural waters.

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As pointed out by our colleague, Dr. L. Provasoli (1961), the heterotrophic capacities of algae are very imperfectly known. This is underscored by recent studies of the green flagellate *Chlamydomonas mundana* as a dominant in sewage lagoons in the Imperial Valley of California (Eppley and MaciasR, 1962); other than that it prefers acetate among the few substrates tried; its heterotrophic capacities are unknown. More unexpectedly, some strains of the photosynthetic bacterium *Rhodopseudomonas palustris* use benzoic acid anaerobically as the reductant in photosynthesis (Scher, Scher, and Hutner, 1962), narrowing the gap between the photosynthetic pseudomonads and the ubiquitous pseudomonads so often represented among bacteria attacking resistant substrates (*eg.*, hydrocarbons) as well as highly vulnerable substrates. For the widely studied, strongly heterotrophic photosynthetic flagellates *Euglena gracilis* and *E. viridis*, common in sewage, no specific enrichment procedure is known, meaning that their ecological niches are unknown but laboratory data provide hints.

The increasing use of oxidation ponds would in any case urge a greater use of scaled-down ecological systems in which development of none of the photosynthesizers present in the original inoculum was suppressed. Conceivably, some of the rare microbes attacking rare substrates--and such microbes are likely to represent a source of degraders of resistant non-biochemicals--are specialists in attacking products of photosynthetic organisms.

Traditionally, the inoculum for a Winogradsky column is a marine or brackish mud (as New Yorkers we would be partial to mud from flats of the Harlem River). Little is known about the effectiveness as inocula of freshwater muds or water-logged soils. It would be valuable to know how complete a column could develop from material from a trickling filter or an activated-sludge plant. A practical issue is: Might the poisoning of a sewage-oxidation system be paralleled by the poisoning of a Winogradsky column, where the poison was mixed with the inoculum for the column? Might the variously colored photosynthetic zones of the column and the aerobic population on top provide sensitive indicators for the performance of a sewage-oxidation system subjected to chemical wastes?

If a particular compound mixed with inoculation mud or sludge suppressed development of the full Winogradsky pattern, one might assume that the compound at the test concentration was poisonous and persistent. Biological destruction of such poisons, if at all possible, might demand a long hunt for suitable microorganisms, then buildup of the culture to a practical scale. This might best be done with illuminated shake or aerated cultures, with the inocula coming from a variety of environments. Optimism that microbes can be found capable of breaking almost all the linkages of organic chemistry is fostered by the study of antibiotics, which include a wealth of previously "unphysiological" linkages--azo compounds, oximes, N-oxides, aliphatic and aromatic nitro and halogen compounds, and strange heterocyclic ring systems. Some natural heterocycles, e.g., pulcherriminic acid and 2-*n*-nonyl-4-hydroxyquinoline, have a disquieting resemblance to the potent carcinogen 4-nitroquinoline N-oxide.

PROTOZOA AS TOXICOLOGICAL TOOLS

A difficult problem is one mentioned earlier: persistence joined with low-grade toxicity to higher animals. Recent developments in the use of protozoa as pharmacological tools show that protozoa can serve as sensitive detectors of metabolic lesions ("side actions"?) of a wide assortment of "safe" drugs. The list includes the "anticholesterol" triparanol (MER/29). Triparanol toxicity manifested itself with several protozoa, including *Ochromonas danica* (Aaronson *et al.*, 1962) and *Tetrahymena* (Holz *et al.*, 1962). Triparanol was not acting simply as an anti-cholesterol for its obvious toxicity to protozoa was annulled by fatty acids as well as by sterols. The connection between the protozoan results and the "side actions" of triparanol -- baldness, impotence, and cataracts -- are of course unclear, but protozoal toxicity might serve as an initial warning that it might not be as harmless as assumed from short-term experiments with higher animals.

The anticonvulsant primidone provides a clear indication of how protozoa can be used to pinpoint the location of a metabolic lesion. Primidone had been known to cause folic acid-responsive anemias. It is therefore easy to find that with joint use of a thymine-dependent *Escherichia coli* and the flagellate *Crithidia fasciculata* reversal of growth inhibition by folic acid and related compounds permitted the charting of interferences with the interconnected folic acid, biopterin, and DNA function (Baker et al., 1962), which amply accounted for the megaloblastic anemias. *Lactobacillus casei*, a bacterium much used in chemotherapeutic research, was unaffected by the drug.

In another instance, where the mode of action of the drug in higher animals was unknown, growth inhibition property of the anticancer compound 1-amino*cyclo*pentane-1-carboxylic acid was reversed for *Ochromonas danica* by L-alanine and glycine, as was the inhibition property of 1-amino-3-methyl-*cyclo*hexane-1-carboxylic acid by L-leucine (Aaronson and Bensky, 1962).

Growth inhibition of *Euglena* by the potent carcinogen 4-nitroquinoline N-oxide was annulled by a combination of tryptophan, tyrosine, nicotinic acid, phenylalanine, uracil (Zahalsky et al., 1962) and, in more recent experiments, the vitamin K relative phthiocol. These N-oxides are of interest because of recent work indicating that perhaps the main way in which the body converts such compounds as the amino hydrocarbons to the actual carcinogens may be by an initial hydroxylation of the nitrogen, e.g., work by Miller et al., (1961). Whether the peroxides in photo-chemical smogs of the Los Angeles type act on hydrocarbons to produce carcinogenic N-oxides is entirely unknown. Leighton (1961) lists an array of peroxy reactions produced by sunlight in polluted air.

Our aforementioned experience with primidone, a ketonic heterocycle, led us to test the sedative thalidomide. It was toxic for *Ochromonas danica O. malhemensis* and *Tetrahymena pyriformis;* this toxicity was annulled by nicotinic acid (or nicotinamide) or vitamin K (menadione) (Frank et al., 1963). We do not know whether a similar protection could have been conferred on human embryos or polyneuritis in the adult.

Many widely used herbicides of the dinitrophenol type are powerful poisons for higher animals. We do not know how sensitive protozoa would be for detecting their persistence. However, since somewhat similar thyro-active compounds can be sensitively detected by their exaggeration of the B_{12} requirement of *Ochromonas malhamensis* (Baker et al., 1961), this flagellate might be a useful test object for dinitrophenols and congeners.

The *Paramecium* (and perhaps too the *Tetrahymena)* test for polynuclear benzenoid carcinogens has remarkable sensitivity and specificity (Epstein and Burroughs, 1962; Hull, 1962). This test depends on the carcinogen-sensitized photodynamic destruction of paramecia by ultraviolet light. This test is approaching practicality for air, and there is no reason to suppose it cannot be applied to benzene extracts of foodstuffs and water.

CONCLUSIONS

We have suggested here new procedures for examining the intrinsic toxicity-persistence relationship, using as test organisms the protists represented conspicuously in a Winogradsky column. The new field of micro-toxicology is virtually undeveloped. The urgent need for detecting chronic, low-grade toxicities is evident from many sides. This is not the place for a detailed discussion of the medical implications of this area of research, but it should be emphasized that chronic toxicities and carcinogenesis are related. Conversely, Umezawa (1961) has remarked that most antitumor substances have chronic toxicities and that elaborate testing procedures for toxicity are required to fix the daily tolerable dose; apparently this problem is a central theme in medical as well as pollution research. Inhibition of growth of an array of protozoa is now in practical use as a means of detecting anticancer substances in antibiotic beers (Johnson et al., 1962).

Since the embryos appear to lack the detoxication mechanisms of the adult animal (Brodie, 1962), toxicity for protozoa (which presumably lack these detoxication mechanisms) should be compared with that for the embryo, not the adult, as emphasized by the thalidomide disaster.

There are further limitations on the use of microbes as detectors of toxicity. High-molecular toxins seem inert to microbes, and antihormones (with the exception of anti-thyroid compounds) are generally inert. The main usefulness of microbial indices of toxicity would appear, then, to be for detecting low-molecular poisons acting on cellular targets rather than on cell systems and organs. These are precisely the poisons likely to put out of business a pollution-control installation primarily dependent on microbial activity.

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THE ENVIRONMENTAL REQUIREMENTS OF FRESH-WATER PROTOZOA

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In "A Study of Some Ecologic Factors Affecting the Distribution of Protozoa," Lackey (1938) discussed much of the existing literature regarding the environmental requirements of the Protozoa and presented some thoughts of his own based upon an extensive series of species determinations from a variety of habitats. It is not my intent to review for this brief presentation the literature bearing on this subject between 1938 and 1962, but merely to call attention to the fact that no major change in thinking has developed in this interval.

Most protozoologists agree that fresh-water Protozoa have a truly cosmopolitan distribution. For example, Bovee (1957) notes the similarity between populations of Protozoa in the Savannah River Basin in North America and the Amazon Basin in South America. However, the most convincing indication of cosmopolitan distribution is the free and successful use of keys to species, such as those of Kahl, Pascher, Gojdics, or Skuja, in all parts of the world. Despite the possibility that a particular species might occur in any part of the world, there is general recognition that it will do so only when specific environmental conditions exist. These conditions may cover a wide range for certain species such as *Stylonychia mutilis* (Muller), which occurs in fresh, brackish, and salt water; or may be quite narrow as is apparently the case for *Stokesia vernalis* Wenrich, which appears only rarely and under almost identical conditions. Thus, the frequency of occurrence may vary from a single record for many species to an almost monotonous regularity for a few species. Even the most ubiquitous species may be dominant in a protozoan population for a relatively short period of time as shown by Wang (1928), Bamforth (1958), and others. Thus, in a natural environment constant replacement of the component species of a protozoan community is a normal event. There is ample evidence that this succession is due not only to variations in pH, dissolved oxygen concentration, temperature, and the like, but also to the direct effect of one species upon another. This interaction includes competition, predation, and the production of substances by a species necessary for the growth of another or which repress the growth of another.

Although it is evident that at least some species may acclimate to conditions beyond their normal range of tolerance (Dallinger, 1887; Finley, 1930; Lackey, 1938; and others), it has not been established that this may be accomplished in the face of the competition and changing environmental conditions in normal streams or lakes. However, individual Protozoa may be found in all sorts of environments from hot springs to snow banks indicating effective natural selection over long periods of time. It is also evident that certain environments may contain only comparatively few species while others may contain an impressive diversity of species suggesting that optimum environmental conditions are also operative at the population level. Since present evidence indicates that it would be both impossible and undesirable to satisfy the environmental requirements of specific protozoans under natural conditions, it appears that the best means of determining whether or not the environmental requirements of fresh-water species are being met is by evaluating the entire protozoan community (in the general sense of the term rather than the restricted use implying a fixed association of species) and the environment in which it occurs. The three most commonly used means for such evaluation are (1) use of indicator species according to a saprobic system, (2) by quantitative determination of the density of organisms, (3) by determining the diversity of species. Ironically, the assumption is often made that one must choose between the various means of evaluation rather than use every means available. Obviously use of several criteria provides a firmer base for evaluating an effect than does use of a single one. This comment is not intended as a criticism of formal systems of evaluation, but only of the rigid use of a single system.

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Many species of protozoans are excellent indicators of specific conditions. Information of this type from both field observations and laboratory cultures is constantly furthering our ability to characterize an environment from the nature of the species inhabiting it. On the other hand, the requirements of the majority of species commonly encountered in fresh water are not clearly defined.

In order to outline the problems existing in the characterization of protozoan populations and their environments, an analysis has been made of the results obtained by the Academy of Natural Sciences of Philadelphia river-survey team. The data were obtained from a series of 202 areas in various rivers and streams mostly in the United States, but in a few cases from other parts of the world. All of these areas were classified as healthy or semi-healthy according to the system described by Patrick (1949, 1950). Although most of the sampling took place in the spring, summer, and fall, a number of winter samplings are included in this total. Each sampling area included approximately 100 to 300 yards of stream or river from bank to bank, and commonly included a riffle, slack water, and a pool area. Collections were obtained in the morning from all common habitats, such as algal growths, mud surfaces, submerged roots, etc., in both shaded and sunlit areas, and were placed in half-pint screw-top jars leaving a half-inch air space above the water surface. These samples were immediately carried to the field laboratory where identifications were made from living specimens. Generally, about 20 half-pint samples were collected, and approximately a dozen different examinations were made from each jar. The samples were first examined under a low-power lens to determine the general structure of the population. More critical and detailed examinations followed. Most samples were taken from the meniscus and the bottom of the jar both toward and away from the principal source of light, although an effort was made to insure that attached species (to twigs, leaves, algal filaments, etc.) were not overlooked. Before the sampling began, several concentration techniques were tried and discarded because many species were destroyed or injured and many streams had a comparatively heavy silt load which interfered with both concentration and examination. Only those species with a density of at least six individuals or more per drop of water from at least one sample were recorded. Furthermore, no samples of the plankton are included in this report, but only those species in association with the substrate. Nearly 1200 species were identified* in the course of these studies, of which approximately 25 percent occurred in four or more of the areas sampled. In other words, approximately 75 percent of the species occurred in three or less of the areas sampled. The 1200 species represent only a fraction of those described. For example nearly 6000 species of ciliates alone, most of which are free-living, have been described to date according to Corliss (1961, p. 23). Three-quarters of the 1200 species recorded in this series of samples occurred less than 1.6 percent of the time. For virtually all of these species the significance of the presence or absence of a single species would be quite difficult to determine. The inhabitants of a number of the areas studied were classified according to their saprobian designation when this was known. For many of these species no information was available. For those species with specific saprobian designation, some sampling areas had excellent agreement in species composition, although the greater number did not. In short, for species which are rare or uncommon, present information does not always permit accurate designation of overall environmental qualities either on the basis of single species occurrence or groups of species.

There remains to be considered the 25 percent of the species which occurred four or more times in the 202 areas sampled. Of these, only 20, or roughly 6 percent, of those found four or more times occurred in at least 25 percent of the areas studied. The number of areas in which each of these 20 species occurred was as follows: 125, 87, 80, 69, 67, 66, 64, 64, 62, 60, 60, 60, 55, 53, 52, 49, 49, 48, 47, 47. Naturally these ubiquitous species occurred together quite often. In an effort to determine whether or not pairs of these species occurred together more frequently than would be expected from chance alone, an association matrix was made for the 20 sampling areas where these species were most common. A Chi-square test of significance was run on the 190 possible associations of species pairs, and of these, 44 occurred together more frequently than expected from chance alone at the 5 percent level of confidence. Also, when the Chi-squares for all of the paired associations were summed, the result showed a highly significant divergence from chance expectation. Comparison of the expected and observed frequency distributions of Chi-square values showed that the divergence was due to the aforementioned large number of significant associates at the 5 percent level of confidence. An examination of the data indicated that associations of three or more species also existed. The fact that these species were able to occur together suggested similar environmental requirements and that the environment might be the major factor producing these associations. To check this possibility the environmental data were processed with a computer and information was obtained for each of the 20 most common species throughout the entire series of occurrences. An example of the grouping of this information for a single species is given in Table 1. Note that species number 42000 occurred 86.11 percent of the time within the phenopthaline alkalinity range of 1.0 to 5.0 ppm, and that the pH range for 97 percent of the records was between 6.5 and 9.0. When the results for the 20 most common species were compared, three factors were noted: (1) pairs or larger groups of associated species always had virtually identical ranges of environmental conditions; (2) these species always tolerated rather broad ranges of environmental conditions; and (3) having identical ranges of tolerance to the chemical and physical environment does not insure that species will be associated more often than would happen by chance alone, since only 44 pairs out of 190 were associated significantly despite the fact that all species had broadly overlapping environmental ranges. In fact, three cases of negative association were found and in each case the environmental characteristics were almost identical. Since only the general environmental characteristics were determined, it must be assumed that competition or materials present in extremely low concentrations may often be critical in these negative associations or that the low number occurring in this series was due to chance alone.

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* Over half of the identifications were made by the authors; the remainder were made by Drs. Mary Gojdics, Ralph Wicterman, Samson McDowell, Jr., Stuart S. Bamforth, and J. Russell Gabel.

At this point, a consideration of the relation of the sampling method to the results is essential. The most important factor is probably the omission of all species below the required density. Unless several protozoologists were to work on a single sample at the same time, identification of these low density species would be impractical because no preservation method is suitable for the entire community and the structure of the community in the unpreserved sample jar changes due to death and division. The low density species may be (1) a species formerly abundant but now in declining numbers, (2) a species increasing in abundance but not yet of sufficient density to be recorded, (3) a species that has persisted at a low population density for a considerable period of time. The first may have produced metabolic products or other substances which affect the population size of the second or vice versa. The second most important factor is probably species with low densities of large size individuals which may represent a greater mass of protoplasm than a small species with a sufficient number of individuals to be recorded. For example, a single *Spirostomum ambiguum* has a volume equal to many *Bodo caudatus*. Perhaps an extension of the system proposed by Šrámek-Hůsek (1958) would permit weighting of these size differences. It would then be possible to determine the relative importance of the quantitative and qualitative relationships between species.

Thirdly, samples of water for chemical analysis were usually composited from several points in each sampling area. Thus, they represent the macro-chemical-physical environment of the protozoan population but not the microenvironment for each component species.

The importance of low density species as reserve components has also been demonstrated by Wallace (in Cairns, 1956), who has shown that variation in temperature will alter the structure of a mixed algal population consisting of diatoms, greens, and blue-greens. She has further shown that increased temperature will result in a dominant blue-green population although originally these algae represented a minor portion of the population. In addition, the diatoms, although virtually absent when the blue-greens are dominant, again became dominant when the temperature was lowered to a range favorable to them. It is quite probable that large numbers of species of Protozoa in a natural environment at low densities form the reserve components which make possible and perhaps, in some cases, even cause the constant shifts in dominant species composition which are typical of a natural fresh-water protozoan community. Thus, parameters for the environmental requirements of fresh-water Protozoa should take into consideration both the existing community of species, with comparatively large numbers of individuals per unit volume, and also those species with relatively few numbers of individuals.

Although the species composition may vary constantly, Patrick (1961) has shown that the number of collectable taxa of the various major groups of aquatic organisms (including Protozoa) remains reasonably similar. More specifically, the number of taxa usually does not vary more than 33 percent from the mean for that group. Patrick states, "Perhaps the reason for this similarity of numbers of taxa of a group of organisms in these areas of natural streams is because there are a finite and similar number of habitats or niches for species occupancy. The numbers of taxa which are available to live in the area under consideration are greater than the number of niches." Thus, as environmental conditions change, new species are able to compete successfully with the dominant species occupying niches and in turn become dominant only to be replaced with the next change in conditions.

To establish the environmental requirements of fresh-water Protozoa, the following factors appear to be of primary importance: (1) chemical and physical standards would either be so broad that they would be meaningless or else they would be inapplicable in view of the wide regional variation in these characteristics of fresh water and even in the seasonal variation of a single stream; (2) constant replacement of both species and entire protozoan

Table 1.

SPECIES NUMBER 42000 PERCENTAGES

RANGE	0	1.0	5.0	10.0	20.0	40.0	80.0	160.0	320.0	OVER			TOTAL
ALKALINE P			86.11	5.56	2.78		5.56						100.0
RANGE	0	5.0	10.0	20.0	30.0	40.0	50.0	100.0	150.0	200.0	550.0	OVER	TOTAL
ALKALINE MO			1.45	5.80	10.14	8.70	4.35	44.93	11.59	8.70	4.35		100.0
RANGE	0	1	3	5	10	50	100	500	2500	10000	15000	OVER	TOTAL
CHLORIDE			8.70	10.14	14.49	43.48	10.14	1.45	10.14	1.45			100.0
RANGE	0	1.0	5.0	10.0	20.0	30.0	40.0	50.0	75.0	100.0	200.0	OVER	TOTAL
CO_2			40.00	60.00									100.0
RANGE	0	1.0	3.0	5.0	7.0	9.0	11.0	15.0	18.0	20.0	OVER		TOTAL
D.O.			3.17	6.35	15.87	44.44	28.57	1.59					100.0
RANGE	0	.01	.03	.05	.07	.1	.4	.7	1.0	5.0	10.0	OVER	TOTAL
FE		18.18	12.12	6.06	12.12	9.09	15.15	21.21	6.06				100.0
RANGE	0	5	10	50	100	150	200	500	1000	2500	5000	OVER	TOTAL
HARDNESS				18.84	24.64	7.25	26.09	18.84	4.35				100.0
RANGE	0	3	10	30	50	75	100	200	450	1000	2000	OVER	TOTAL
Ca		5.80	11.59	27.54	31.88	15.94	4.35	2.90					100.0
RANGE	0	3	10	30	50	75	100	200	450	750	1000	OVER	TOTAL
Mg		18.84	23.19	39.13	7.25	8.70	1.45	1.45					100.0
RANGE	0	.001	.009	.03	.05	1.0	10.0	20.0	40.0	60.0	OVER		TOTAL
NH_3-N		18.57	8.57	25.71	11.43	28.57	4.29	1.43		1.43			100.0
RANGE	0	.001	.007	.013	.03	.07	.2	1.5	3.0	7.0	OVER		TOTAL
NO_2-N		13.85	60.00	12.31	1.54	7.69	1.54	3.08					100.0
RANGE	0	.001	.007	.013	.03	.07	.2	.7	1.5	3.0	7.0	OVER	TOTAL
NO_3-N			2.86		5.71	14.29	50.00	15.71	5.71	4.29	1.43		100.0
RANGE	0	3.0	4.5	5.0	5.5	6.0	6.5	7.5	8.0	9.0	9.5	OVER	TOTAL
pH				1.45			2.90	34.78	33.33	26.09	1.45		100.0
RANGE	0	.001	.005	.01	.05	.1	.5	1.0	5.0	10.0	OVER		TOTAL
PO_4		2.86	12.86	7.14	25.71	21.43	17.14	1.43	5.71		5.71		100.0
RANGE	0	.001	.01	.05	.1	.5	1.0	3.0	6.0	12.0	48.0	OVER	TOTAL
SiO_2						1.45		10.14	31.88	40.58	15.94		100.0
RANGE	0	1	10	30	60	90	120	240	480	960	1500	OVER	TOTAL
SO_4		2.90	20.29	37.68	23.19		2.90	7.25	4.35	1.45			100.0
RANGE	0	4.0	12.0	17.0	20.0	23.0	28.0	30.0	35.0	40.0	OVER		TOTAL
TEMPERATURE				3.08	13.85	26.15	47.69	3.08	1.54		4.62		100.0
RANGE	0	10	25	50	100	500	1000	2500	5000	7500	10000	OVER	TOTAL
TURBIDITY		13.04	26.09	20.29	36.23	4.35							100.0
RANGE	0	.1	.5	1.0	5.0	10.0	20.0	40.0	80.0	OVER			TOTAL
B.O.D.		12.00	18.00	16.00	34.00	8.00	10.00	2.00					100.0
RANGE	0	.06	10.0	25.0	OVER								TOTAL
TOTAL P													100.0
RANGE	0	.001	.01	.02	OVER								TOTAL
TOTAL N													100.0

communities is the rule rather than the exception; (3) the large species reservoir which exists in inactive stages or in active stages below the population density, easily detectable by the usual examination techniques, is necessary for the constant replacement of species; (4) a limited number of niches is available in natural stream situations resulting in intense competition and constant replacement of species with shifts in the chemical and physical environment.

The requirements of Protozoa may be stated as those conditions which permit the protozoan population to remain within the range of species diversity found to be normal for the environment in question. Obviously, considerably more data will be required before the normal range or variation for the various types of aquatic environments becomes reliably established. Furthermore, since Protozoa are part of a larger aquatic community one should evaluate this first, together with the general chemical and physical environment, before proceeding to any specific group evaluation. These multiple lines of evidence would thus provide a much firmer basis for determining the degree to which the requirements of the aquatic biota are being met.

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RELATIONS OF PLANKTONIC CRUSTACEA TO DIFFERENT ASPECTS OF POLLUTION

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In 1909, Kolkwitz and Marson classified the pollution of waters into saprobiological zones (reported by some succeeding authors as saprobiological degrees). They supposed that various saprobiological zones differ by the extent to which organic matter that is introduced by pollution is mineralized. They considered, for example, *Daphnia pulex* de Geer, *D. magna* Straus, and *D. longispina* O. F. Müller as b-mesosaprobic (to some extent also α-mesosaprobic), *Daphnia hyalina* Leydig with the ssp. *D. h. galeata* Sars, and *Daphnia cucullata* Sars with all its varieties, as oligosaprobic. They regarded *Bosmina longirostris* Müller as both oligosaprobic and as β-mesosaprobic. From this it follows that most of the species can develop in different saprobiological zones, so that the proper use of these animals as indicators depends more or less upon experience. Zelinka, Marvan, and Kubiček (1959) developed a more objective procedure, the principle of which can be explained by the following example.

Table 1 shows the saprobiological values of different species of Cladocera. For example, *Daphnia magna* has the value 4 in the α-mesosaprobic zone and the value 6 in the polysaprobic zone. (The sum of the values of all zones is, for each species, 10.) The saprobiological evaluation of a sample from a pond in Table 2 is performed by expressing the frequency of the species in the sample in absolute numbers or in relative ones (in percents), and by multiplying the frequency by the saprobiological values in question. In this way we find the sum of these products in the different saprobiological zones. The sample in question is classified into the saprobiological zone that possesses the greatest sum. If some doubt arises, then greater weight is attributed to species occurring in their proper frequency (indicated in numbers according to the Braun-Blanquet scale in the last column of Table 1).

But even if this procedure is used, uncertainty remains in regard to the objective measure of the individual zones by the presence of the chemical substances. The BOD and COD are most commonly used as chemical measures of pollution. However when comparing bodies of water in which there was a twofold difference in mean BOD and COD, we could not find a definite difference in zooplankton composition. The most common Cladocerans in the plankton, both in Hořejší pond in 1957 and Klíčava reservoir in 1960, were *Bosmina longirostris* Müller, *Daphnia cucullata* Sars, *Daphnia longispina* Müller, *Diaphanosoma brachyurum* (Lievin) and *Ceriodaphnia pulchella* (Sars). The mean BOD of the surface water of the pond was 7 and of the reservoir 2 milligrams per liter O_2. In 1960, the COD of the surface water from the Klíčava reservoir was 12.8 and from the Slapy reservoir, with practically identical composition of zooplankton, 48 milligrams per liter O_2. On the other hand, *Daphnia pulex* de Geer prevailed in ponds where the BOD and COD of the surface water were intermediate between these extremes (the mean BOD of these ponds ranged from 3.3 to 3.9 and the COD from 25.2 to 29.8 milligrams per liter O_2). This would imply that neither of these properties is directly related to any selective factor that could be responsible for the different development of plankton in Crustacea. This makes it necessary to review the findings on the influence of different injurious polluting substances upon the different species of zooplankton.

One of the most investigated aspects is the effect of the low oxygen content upon zooplankton. In lakes with a clinograde oxygen curve, with a strong deficiency of oxygen in the hypolimnium, planktonic crustaceans are frequently observed in samples with only a few tenths of milligram per liter of oxygen. It is probable that the cladocerans observed are not

Table 1. EVALUATION OF THE OCCURENCE OF PLANKT. CLADOCERA IN SAPROBIOLOGICAL ZONES (Zelinka and others, 1959)

Species	Saprobiological zones[a]						
	bos	aos	bms	ams	ps	hs	frequency
Bosmina longirostris (O.F.M.)	1	4	4	1	-	-	5
Ceriodaphnia pulchella Sars	1	4	5	-	-	-	4
Daphnia cucullata Sars	+	4	6	-	-	-	4
Daphnia longispina O.F.M.	1	2	4	3	-	-	5
Daphnia magna Straus	-	-	+	4	6	-	5
Daphnia pulex de Geer	-	-	+	5	5	-	5
Diaphanosoma brachyurum Liev	+	5	5	-	-	-	4

[a] bos = beta-oligosaprobic zone
bms = beta-mesosaprobic zone
ps = polysaprobic zone
aos = alpha-oligosaprobic zone
ams = alpha-mesosaprobic zone
hs = hypersaprobic zone

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living under these conditions for a long period of time, because they do not exhibit the red color that has been noted in specimens from very productive ponds, where, throughout some periods, low concentrations of oxygen occur, especially during night hours. Fox (1951) and his collaborators have shown that this color is caused by hemoglobin, which increases the survival time of *Daphnia* species in semianaerobic conditions.

In small ponds, where, under the ice cover in winter, the concentration of oxygen drops to zero, and sometimes even hydrogen-sulphide is present in concentrations up to several milligrams per liter, only some species of Cyclopidae, for example, *Cyclops viridis* (Jurine) and *Cyclops strenuus* (Fisher), have been recorded (Prokešová, 1959). Under such conditions, the species cannot develop until the moment when, after the disappearance of ice, oxygen appears in the water again.

Table 2. POND OSTROVEC U POZDATINA 26.7.1949

Species	Saprobiological zones[a]						
	Frequency in %	bos	aos	bms	ams	ps	hs
Daphnia pulex de Geer	52	-	-	-	260	260	-
Daphnia magna Straus	45	-	-	-	180	270	-
Diaphanosoma brachyurium Liev	1	-	5	5	-	-	-
			5	5	440	530	-

[a] bos = beta-oligosaprobic zone
bms = beta-mesosaprobic zone
ps = polysaprobic zone
aos = alpha-oligosaprobic zone
ams = alpha-mesosaprobic zone
hs = hypersaprobic zone

A pH value as low as 4.5 has been found in bodies of water where the planktonic crustaceans were represented only by the genus *Ceriodaphnia*. In ponds with a still lower pH, Cyclopidae also are rare or absent and zooplankton is represented merely by Rotatoria. This low pH value may result not only from pollution by industrial wastes, but also from improper cultivation of the Norway spruce. In three Bohemian mountain lakes of glacial origin where, at the end of the past century, a relatively rich development of *Daphnia longispina* and *Bosmina* species occurred (Frič and Vávra, 1898), none of the species mentioned has been found at present. The original beach forest was, in the second half of the last century, replaced by a Norway spruce monoculture, which is said to acidify the soil. Because in the past century the pH of the lake water was not measured, the low value of pH recorded at present cannot be proved to be merely of recent origin. This could be done, however, in areas where the change in forest cultivation has taken place quite recently. K. Berg and I. C. Petersen (1956) explain the disappearance of *Holopedium gibberum* Zaddach in Lake Gribsø in Denmark during the last century by similar events.

Daphnia magna Straus is commonly used in tests for toxicity, but, as far as is known, no differences in the susceptibility to toxicants of different species have been investigated. From observations on lakes and ponds treated with insecticides and other toxic substances for the destruction of coarse fish, Cladocera are reported to be most affected of all the zooplankton groups (Almguist , 1959).

From this brief outline of effects of abiotic factors, it follows that, so far as is known, abiotic factors best act selectively when reaching extreme values. This may be inferred also from data on distribution of some species as, for example, of *Daphnia pulex* de Geer, recorded both from clear mountain lakes above the limit of the dwarf pine with water very poor in dissolved substances (Litynski, 1913) and from small village ponds with a very high content of inorganic and organic matter (Langhans, 1936; Wagler, 1937). It is to be pointed out, however, that there exist waters with intermediate positions where not a single specimen of *Daphnia pulex* can be observed--mountain lakes, for example, below the limit of the dwarf pine, that may lie in the same valley as do lakes where *Daphnia pulex* has been recorded (Litynski, 1913).

From this it may be concluded that the presence of different species in different bodies of water depends on the biotic environment. To examine this possibility, two sets of experiments were stated.

One set, worked out to a great extent by Hrbáčkova (Esslova, 1962 and in press), consisted of cultivations of individual specimens of different *Daphnia* species in 100-milliliter flasks, daily filled with about 50 milliliter of water enriched with algae cultivated in the laboratory, or with 50 milliliter of surface water from two reservoirs. The cultivated specimens were measured after each molt. In the experimental series with surface water from the Slapy reservoir, where about 0.1 milligram per liter fresh weight of nannoplanktonic algae and about 10 milligram per liter fresh weight of blue-green algae (mainly *Aphanizomenon)* occured, three *Daphnia* species were examined: *Daphnia pulex* from a small village pond rich in nannoplankton, *Daphnia pulex* from a large pond poor in nannoplankton (according to the morphological features found to be identical with *Daphnia* Forbes Hrbáček, 1959), and *Daphnia hyalina* Leydig from the Slapy reservoir. *Daphnia hyalina*, in addition to *Daphnia cucullata*, is common in the Slapy reservoir, *Daphnia pulicaria* very rare, and *Daphnia pulex* has not been found up to the present. The experiment showed that *Daphnia pulex* cannot reproduce in the daily renewed Slapy water; the newly born individuals, thus kept, died after 3 to 4 molts with symptoms of total exhaustion. *Daphnia pulicaria* and *Daphnia hyalina* showed a slower growth in the Slapy water as well as a larger number of molts to maturity than did the control individuals, which were kept in the Slapy water, enriched with laboratory-cultured *Scenedesmus* and *Chlorella*. The slower growth was in terms of the length increment of each molt and also of the period from the release of the young individuals (the so-called neonatae) to their maturity (the so-called primiparae). This period was prolonged from the five days normal in the controls to at least eight days with respect to the experimental individuals. The smaller relative increment can be seen (Figure 1). That the lower increment is caused by the low quantity of food, not by its quality, is shown by the great improvement in the growth of cultures that were enriched about five times with nannoseston by centrifugation (dotted line). In experiments with *Daphnia hyalina*, a greater variability has been observed, but the results were the same. The average number of eggs, under experimental conditions, was recorded in *Daphnia hyalina*, namely one-half of that found in *Daphnia pulicaria*. *Daphnia pulex*, when plentifully fed, reached maturity by about one-tenth of the whole period earlier than did *Daphnia pulicaria*. In the water of another reservoir, which was about three times richer in nannoplankton than the Slapy reservoir, the species that first reached maturity was *Daphnia longispina*, which does not occur in the reservoir itself. *Daphnia*

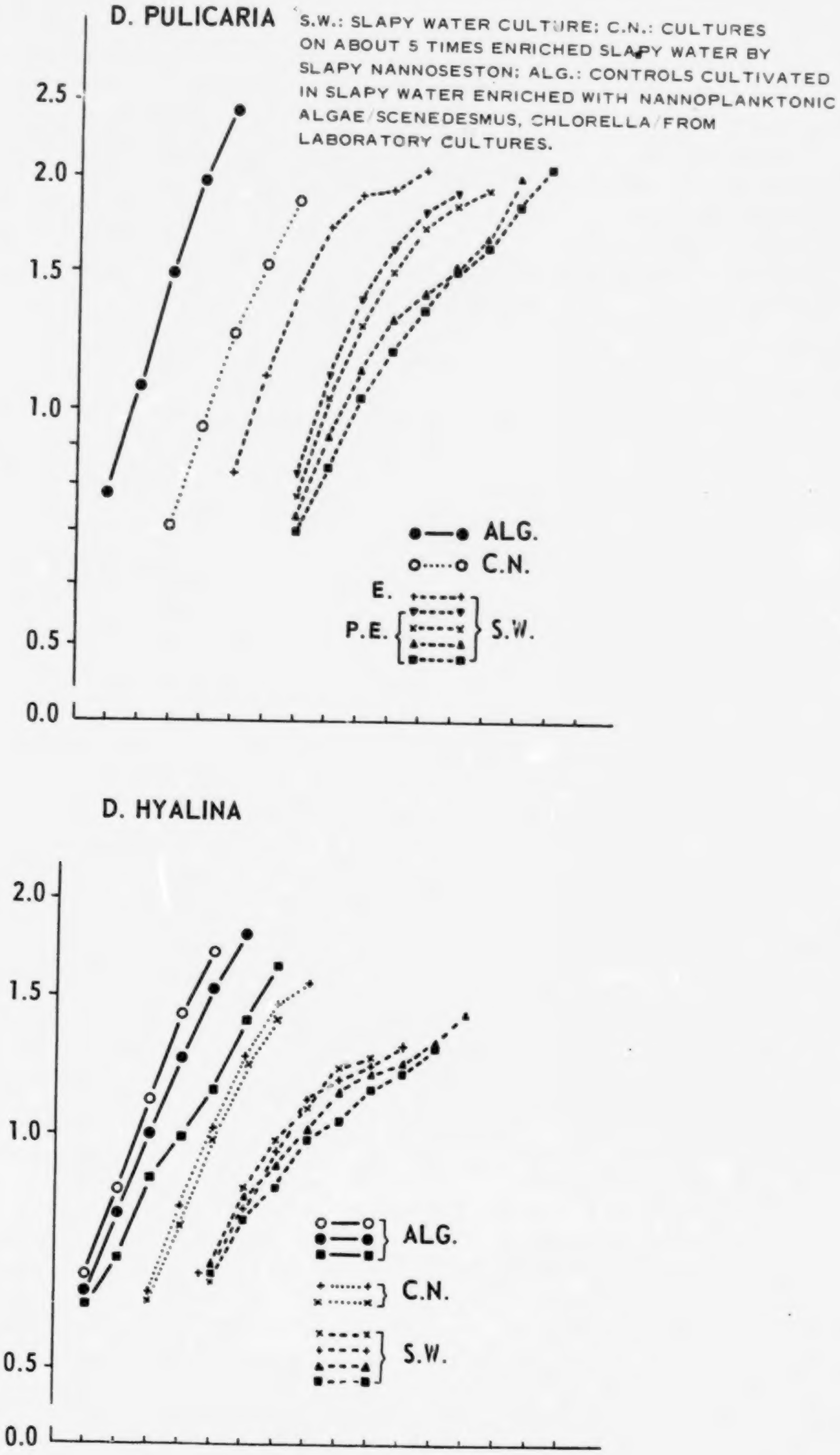

Figure 1. The logarithmic scale of the length is plotted on the Y-axis, the number of molts on X-axis. Individual points indicate the average length of several individuals; full lines show the growth of the control individuals, broken lines, the growth of the experimental individuals.

pulicaria, *Daphnia hyalina*, and *Daphnia pulex* all reached maturity later. From this it follows that the results of both experiments do not explain the predominance of *Daphnia hyalina* in both reservoirs as caused by food conditions; on the contrary, the food conditions may be responsible for the exclusion of some species.

The relative increment, from neonatae to primiparae, under both experimental and control conditions, was found smaller in *Daphnia hyalina* from both reservoirs than in *Daphnia pulicaria* and *Daphnia longispina* in the experiments. On examining the relative increment of different species and different clones at plentiful feeding at 20° C (Hrbacek and Hrbáčkova, 1960), it was found that species from bodies of water without a fishstock or with a low one (for example, *D. curvirostris* and *D. pulicaria)* showed invariably larger relative increments than did species (for example, *D. cucullata)* from bodies of water with a numerous fish stock. In some species, as in *Daphnia hyalina* and *Daphnia longispina*, clones of different relative increments were found from bodies of water with different fish stocks. We can explain the predominance of *Daphnia hyalina*, and *Daphnia cucullata* in both reservoirs by the assumption that the fish stock is the main factor responsible.

The other set of experiments was performed on small backwaters (Hrbáček, 1962) where stunted populations of coarse fish occurred and where, of the planktonic cladocerans, *Bosmina longirostris* predominated, and the genus *Daphnia* was represented by *Daphnia cucullata*. After the destruction of the fish stock, *Bosmina* and *Daphnia cucullata* entirely disappeared and were replaced by populations of either *Daphnia hyalina* or *Daphnia longispina*. The most interesting experiment of this set concerned the destruction of the fish stock from one of the backwaters of about the same area, both drained by the same periodic outlet from a village pond (Hrbáček et al., 1961). The destruction of the fish took place in late summer of 1956, in the backwater nearer to the village pond called Poltruba. The next year, *Daphnia hyalina* (exhibiting large relative increments) predominated among the cladocerans in the Poltruba, whereas *Bosmina longirostris* was the leading species in the other backwaters. It is interesting that both the BOD and COD were found invariably lower in backwaters without fish, where *Daphnia hyalina* occurred, although the renewal of the water was, at least in some periods, very intensive, lasting at times merely several days, so that this difference cannot be attributed to external factors.

From the above sets of experiments, it may be safely concluded that the biotic environment is a selective factor, promoting one species while suppressing the others. It can be assumed also that pollution, in most cases, does not act directly upon different species of zooplankton, but that it does change their biotic environment. A part of the changes in zooplankton, attributed to pollution, may be owing instead to the changed nutritional level that results from the development of bacteria or algae. Other changes are owing to changes in the composition of the fish stock, since the oxygen deficiency, one of the most common consequences of pollution, affects predators more seriously than some species of coarse fish. If this is true, then the situation may be improved, at least in some cases, by proper fish management, namely by making the necessary arrangements against the undesired propagation of coarse fish.

From our experiments, the need seems to be for a more thorough knowledge of the physiological differences among species and genera that underlie the differences in their distribution. Without this knowledge we shall not succeed in giving a thorough explanation of the events observed, or our explanations will be based on poorly defined factors.

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THE BIOLOGY OF THE TUBIFICIDAE WITH SPECIAL REFERENCE TO POLLUTION

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The title of this paper is ambitious in view of the present lack of information on the biology of the aquatic oligochaetes. Most of the literature on these worms is largely taxonomic, but owing to a good deal of confusion about valid characters and no recent revision, it has been difficult for ecologists to identify tubificids with any confidence. Most of the biological data that are available refer specifically to *Tubifex tubifex* and *Limnodrilus hoffmeisteri* although I suspect that we may not always place too much reliance on the names used. Having recently attempted to sort out the taxonomy (Brinkhurst, 1963), I feel we are now in a position to point the way to future work on this family, which will then be of use in the detection and assessment of pollution of various kinds in inland waters.

It seems to me that there are three basic approaches to the use of macroinvertebrates in relation to pollution (by which I mean not only pollution by organic matter but also the effects of poisons, inert solids, insecticides, and so on). These I would describe as: (1) the establishment of physical-chemical tolerance limits for individual species, (2) the search for indicator species, the mere presence or absence of which can be used to categorize the water concerned, (3) detailed analysis of community structure by all available parameters, including biotic and abiotic factors, consistent with the time, staff, and facilities available. My colleague, Dr. Hynes, has recently published a book in which the third approach is dealt with in detail, and he will show how sensitive a tool this can be in his address to this symposium. My own limited experience in the field of river pollution has been as an individual consultant, usually working alone with access to only simple facilities for chemical analysis. In this work I have found that an analysis of the bottom fauna of a series of stations with closely comparable substrata gives a clear indication of the source, nature and extent of any "pollution" (if I may be allowed to beg the question of a definition of that word). I now wish to summarize our knowledge of the Tubificidae in relation to these three approaches, and I hope that in so doing I will have indicated the lines of future research that seem to me to be most profitable to pursue. I must here express my thanks to thos who have supported my tour this summer (includin the Royal Society, The American Philosophical Society, The American Society of Limnology and Oceanography, and, not least, Dr. Ruth Patrick). Fro this tour I hope to lay the foundations of a taxonomi revision of the North American species of the family.

1. The tolerance limits of individual species t factors of the environment.

The first difficulty encountered in studying th distribution of the Tubificidae is that the number of both species and individuals seem to vary in manner apparently unrelated to any obvious chemica or physical factor. Many species occur in rivers streams, pools, and lakes, of all sorts and al conditions, mostly inhabiting mud but demonstrating no clear habitat preferences. The distributio of species in lakes makes no sense in terms o abiotic factors of the environment (as I will illustrat in a paper to be read at the Limnological Congres at Madison), and the same is true of worms collecte from rivers. Very different localities may, however support surprisingly similar populations. As a example of the absence of any clear pattern of th abundance of species in relation to substratum, depth, or emergent vegetation, one may examin Table 1. As this represents an analysis of subsamples of up to 50 worms from qualitative collections, we can only regard these data as giving a rough estimate of the abundance of the species at each station. A second survey, carried out 2 years later by C. R. Kennedy also failed to find any pattern in the distribution of the worms, but did find that the relative abundance of the species, expressed as the percentage composition of the total worm population from all samples, seemed remarkably the same as that observed in the first survey (Table 1). The same species were collected (together with a single specimen of *Peloscolex velutinus)* in much the same numbers, but not necessarily in the same places, as before. The one clear correlation was between the occurrence of *Branchiura sowerbyi* and a warm water effluent from a power station (maximum temperature 25° C).

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In contrast, Ditton Brook is a small stream, organically polluted by a piggery and then blanketed with coal particles from the washing plant of a nearby mine. The relative abundance of *Tubifex tubifex* and *Limnodrilus* species in the tubificid fauna varies quite markedly at different places and also at the same place over a period of time (Tables 2 and 3). This sort of observation was made by Dr. Purdy, a former worker in this institution (S.E.C.), but I have not included his work in this review as I have yet to see his unpublished data which might modify or extend what little published information he left us.

It is not, therefore, easy to decide which factors of the environment are important and require detailed study. Furthermore, the tolerance levels for cocoons, young worms, mature worms, and breeding individuals may differ, tolerance limits may vary seasonally, and interactions between environmental factors will undoubtedly occur; and so, in practice, we may prefer to delay the establishment of criteria by experiment until the field observations are better developed.

Table 1. TUBIFICIDAE COLLECTED FROM TRANSECTS ACROSS RIVER THAMES AT READING, BERKSHIRE (April 14 to 16, 1959)

Transect	Yards from south or north bank	Substratum	Vegetation	Depth in feet	SPECIES[a]											
					1	2	3	4	5	6	7	8	9	10	11	12
1	S 1	Gravel	-	1	1	-	-	-	6	-	1	3	-	-	-	-
	5	Stones	-	2	3	10	-	-	3	-	-	-	-	-	-	-
	10	"	-	7	21	6	1	-	10	-	-	-	-	-	-	-
	20	"	-	12	2	-	-	-	-	-	2	-	-	-	-	-
	40	"	-	12.5	-	C	-	-	-	-	-	C	-	-	-	-
	55	Black mud and twigs	-	4	-	C	-	-	-	-	-	C	-	-	-	-
2	S 1	Mud	*Acorus*	1.5	-	-	-	-	C	-	1	C	1	1	-	-
	2	"	"	2	C	8	-	-	1	1	1	-	-	-	-	1
	5	Black, soft mud	*Nuphar*	3	2	20	-	-	8	-	-	-	-	-	-	-
	Middle	Shell gravel	-	10.5	-	2	-	-	2	-	2	2	-	-	-	-
	N 5	Black mud	-	4	2	C	2	-	-	-	1	2	-	-	-	-
	3	Over stiff	-	2	-	2	1	-	6	-	-	6	-	-	-	-
	1	Clay	-	1	2	1	-	-	-	-	-	-	-	-	-	-
3	S 1	Mud	*Acorus, Glyceria*	2	1	6	-	-	-	-	-	-	-	-	-	2
	S 4	Black mud	edge of *Acorus*	2.5	1	C	-	-	-	-	-	-	-	-	-	-
	N 1	(as N Tr. 2)		6	2	C	-	-	C	-	1	2	-	-	-	-
5	S 1	Oily black mud	*Carex, Acorus*	1	5	C	-	4	-	-	-	-	-	-	C	-
	3	" " "	*Nuphar*	2	5	C	4	1	5	-	-	-	-	-	2	-
	5	" " "	"	4	-	C	-	-	-	-	-	-	-	-	-	2
	8	" " "	"	6	-	-	-	-	2	-	-	-	-	-	-	-
	20	Stones	-	8	-	1	4	-	C	-	-	4	-	1	-	-
	N 1	(as N 2)	-	4	7	12	1	-	5	-	-	2	-	-	1	1
Total (C 25)					79	248	13	5	123	1	9	96	1	2	28	6
Percentage composition					12.9	40.6	2.1	0.8	20.1	0.2	1.5	15.7	0.2	0.3	4.6	1.0
" " in 1962 (C. Kennedy)					21.8	34.5	2.6	0.2	28.7	0.6	0.4	5.0	0.2	0.2	2.2	3.0

[a] 1. *Limnodrilus claparedeanus + L. longus.*
2. *L. hoffmeisteri.*
3. *Euilyodrilus hammoniensis.*
4. *Euilyodrilus bavaricus.*
5. *Euilyodrilus moldaviensis.*
6. *Tubifex ignota.*
7. *T. tubifex.*
8. *Psammoryctes barbata.*
9. *Psammoryctes albicola.*
10. *Rhyacodrilus coccineus.*
11. *Branchuira sowerbyi.*
12. *Limnodrilus udekemianus.*

a. Substratum

Valle (1927), Rzoska (1936), Szczepanski (1953), Brinkhurst and Kennedy (1962), and probably other authors have noted apparent correlations between the nature of sediments in lakes and rivers and the species of worms present (or the relative abundance of those species), but these may only be used as pointers to further research in the absence of careful studies of replicate samples from each sediment considered. Della Croce (1955) studied the bottom sediments of Lake Maggiore in some detail, and from July 1953 to May 1954 samples were obtained from five depths in the lake. These were analyzed to provide data on the numbers of the two species present in relation to the nature of the sediments, expressed as the percentage composition by weight of sediment in each of five fractions separated by elutriation. The results were formulated as a general rule: "The number of the Oligochaeta decreases when the percent value of the II fraction increases, an opposite effect being displayed by the fraction IV, while fraction III does not seem to have a definite effect." In practice, this rule holds good for one species *(Peloscolex ferox)* at two of the depths studied, whilst the reverse is true at one depth for *Bythonomous lemani*. There was no correlation at other depths. Therefore it seemed difficult to accept this as a general rule, especially as the analysis was based on single samples, giving us no idea of the variation due to sampling. With the assistance of Dr. P. M. Sheppard (Genetics Department, Liverpool) these results were reanalyzed.

Table 2. ANALYSES OF THE BOTTOM FAUNA PRESENT IN SAMPLES FROM DITTON BROOK[a]

	Transect 1						Transect 2						Transect 3		Transect 4	
	A		B		C		A		B		C		A		A	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Total number of Tubificidae/100 cm^3	121	174	128	118	73	15	17	13	40	97	130	102	0	0	1	0
% *Tubifex tubifex*	64.3	67.2	77.6	52.7	51.1	30.4	14.3	20.8	90.1	82.6	85.3	66.8	0	0	0	0
Total number of cocoons/100 cm^3	60	50	27	25	30	5	4	4	14	64	43	48	2	1	11	15
Total number of *Chironomus* sp. larvae/100 cm^3	0	0	4	3	0	0	0	0	8	5	0	0	32	32	6	2

[a]Numbers given per 100 cm^3 of mud. Four transects were sampled at left bank (A), midstream (B), and right bank (C). Duplicate samples were obtained at each station (A1, A2, B1, B2, etc.). Remaining tubificid fauna chiefly *Limnodrilus hoffmeisteri* and some *L. udekemianus*.

Table 3. PERCENTAGE OF *TUBIFEX TUBIFEX* IN SAMPLES FROM STATION 3A IN DITTON BROOK, 1959-60[a]

Month	1	3	5	6	7	8	10	11	12
% *Tubifex tubifex*	28	42	47	58	33	43	40	33	33

[a]Based on subsamples from qualitative samples only.

We first obtained a single measure of the nature of the sediment in each sample by deriving a mean fraction value from the percentage composition by weight of each fraction (obtained as $\frac{\%I \times I - \%II \times 2 \ldots \%V \times 5}{100}$). It was then found that there was a positive significant correlation between the mean sediment value and the abundance of *P. ferox* at three depths in Lake Maggiore (60 meters, $r = 0.844$ $P < 0.01$; 100 meters, $r = 0.82$ $P < 0.01$; 300 meters, $r = 0.647$ $P < 0.05$) but a non-significant negative correlation at the other two depths. Furthermore, there was a steady shift in the mean sediment value with time (from about 2.3 to 3.1) which occurred at all depths. The mean of these mean sediment values changed steadily with depth, with an abrupt change at the lowest level (30 meters = 2.84; 60 meters = 2.83; 100 meters = 2.82; 200 meters = 2.80; 300 meters = 2.67). In those three stations with a positive correlation between particle value and abundance of *P. ferox*, there is clearly an increase in the population with time. There are three possible interpretations of such a situation, all being more or less reasonable in biological terms. Either the particle size is affecting the number of worms or vice versa, or some external factor (for which we can use time) is affecting both independently. Using partial correlation analysis on the data obtained at 60 meters (where the correlation between particle value and worms is stringent when time is ignored), we find that when the correlation due to time is removed, the correlation between sediments and *P. ferox* abundance is insignificant (r 12.3 = 0.151 with 6 degrees of freedom). Therefore, it would appear that some external agency was increasing the mean particle value and the numbers of *P. ferox* at the same time. We may therefore suppose the particles were becoming lighter with time as particle fraction I consisted of the heaviest particles. There is also what would appear to be a highly significant correlation between the percentage of *P. ferox* as opposed to *B. lemani* and depth, with a critical depth between 100 and 200 M. The percentage of *P. ferox* is given as follows: 30 meters, 74.02 to 75.00; 60 meters, 75.75 to 71.60; 100 meters, 76.44 to 71.65; 200 meters, 45.00 to 48.57; 300 meters, 43.51 to 51.48 percent, indicating another factor important in determining the relative abundance of the worms, such as the distribution limits of a predator or the physical-chemical conditions in relation to the thermocline or the like. If the results of such detailed work (albeit omitting duplicate sampling) cannot establish a direct relationship between worms and sediments, we may view more superficial correlations based on visual estimates with suspicion.

Two species which might well repay detailed study in relation to sediments are *Rhyacodrilus coccineus*, which is found widely in sandy rivers and streams but very seldom in soft mud, and *Aulodrilus pluriseta*, which is usually found in association with very fine silt. I intend to investigate this problem by taking a large number of samples at one time from a constant depth in a lake as soon as possible.

b. Organic matter in sediments

Ravera (1951) studied the populations of benthonic animals in a newly cut-off meander of the River Toce, where the most abundant animals were tubificids (probably *Psammoryctes barbata)*. This showed that "The Tubificidae population density increased from the upstream to the downstream zone of the meander, and everywhere from the beginning to the end of the research period; in the same way the percentage organic matter varied within the sediments." Again we have a correlation, but possibly not proof, of a causative relationship between percentage organic matter and tubificidae alone. Della Croce (1955) found no such correlation between total organic matter and worm abundance in Lake Maggiore. The significant factor here may be that fraction of the organic matter supporting bacterial colonies.

c. Chemical factors

In my paper read at Madison Wis. (Brinkhurst, 1963) I have listed those species that appear to be marine, those which are restricted to brackish water or water of varying salinity in estuaries, and those predominantly freshwater species than can withstand some degree of salinity. It is, therefore, apparent that chloride ion tolerance (probably together with competition from other species) may limit the distribution of some species. In a fast-running stony stream in England with a salinity of 150-250 ppm Cl (maximum 505 ppm) associated with a BOD averaging about 35 ppm (maximum 55.4 ppm) and a percentage saturation with oxygen varying from 10.7 to 88.9 percent, a dense tubificid population was made up largely of *Psammoryctes barbata* together with *Tubifex tubifex*, *Limnodrilus hoffmeisteri*, and *Limnodrilus udekemianus*. *Rhyacodrilus coccineus*, which was common in the main river of which this was a tributary, was absent here; the absence is probably associated with the periodic shortage of oxygen rather than the high salinity figure. *Psammoryctes barbata* has also been recorded more often from rivers in which we suspect a relatively high calcium content, but it is clearly not restricted by relatively high chloride levels in fresh water.

Jones (1938) reviewed the effects of heavy metals on freshwater animals. These showed that water from copper stills was lethal to *"Tubifex"* but that this effect could be dispelled by using amorphous substances of various kinds. Potassium chloride rendered *"Tubifex"* inert, but it recovered upon addition of calcium chloride. Lead nitrate at a concentration of 0.01 N was lethal to *"Tubifex"* in 30 minutes, 0.08 N in 60 minutes, but the addition of lead nitrate to copper nitrate at 0.05 N showed an increased survival up to 0.01 N lead nitrate, but an enhanced toxicity at higher concentrations of lead in the copper solution. These facts are indicative of the complexity of the possible interactions between factors of the environment.

Hynes (1961) has shown that various oligochaetes *Nais pseudobtusa* (determination regarded as uncertain), *T. tubifex*, *Lumbriculus variegatus*, *Eiseniella tetraedra)* became abundant in a small stream subsequent to pollution from sheep dip containing the insecticide BHC, and suggested that this was due not only to an ability to survive the insecticide but also to the absence of predators.

d. Oxygen

There is little information regarding the oxygen requirements of various tubificids. It is well known that these worms contain red blood pigments and that some, at least, can survive when oxygen tensions are very low indeed for considerable periods. Alsterberg (1922), Dawsend (1931) and Harnisch (1935) investigated the physiology of respiration, chiefly with *T. tubifex* if we are to believe the identifications. Eggleton (1931) expressed the view that tubificids were facultative rather than obligatory anaerobes, as have many other workers. Fox and Taylor (1955) showed that pure oxygen was lethal to tubificids, but that newly hatched worms survived best at low oxygen tensions and grew three times as fast as when kept in well aerated water. Berg et al. (1962) have established that *T. tubifex* and *Limnodrilus udekemianus* take up oxygen at a fairly steady rate down to 4 percent oxygen (as percentage of a gas mixture in equilibrium with the water) whereupon the rate falls rapidly to a critical value at about 2.5 percent. Rather comparable results were obtained with two other species, *Euilyodrilus hammoniensis* and *Psammoryctes barbata*. As we would expect, then, some species at least can take up oxygen at very low concentrations.

Differential survival of species in relation to oxygen concentrations is probably implicit in much of what follows in section 3, but we have insufficient data available in the way of continuous records from single localities to define tolerance limits here.

e. Temperature

Berg et al. (1962) have shown that temperature affects the respiration rate of tubificids, and Dawsend (1931) has shown that they withstood anaerobic conditions for 48 days at 0° to 2°C but only 9 days at 18° to 20° C. Mann (1958) and Brinkhurst (1960) have recorded the distribution of *Branchiura sowerbyi* in relation to a warm effluent in the Thames at Reading, which reaches a maximum of 25° C. This is a tropical species that occurs in many botanic gardens and that has escaped to the wild in Europe, not only to warm sites but also to quite normal temperate habitats. Beyond this, however, I can find no references to the effects of temperature on tubificids.

In general, this approach to the subject under consideration is fraught with difficulties, particularly as laboratory studies of individual components of the environment will tell us little about the distribution and abundance of the worms in nature. These studies usually ignore interactions of various components of the environment and the differences between eggs, young and old individuals, as well as seasonal fluctuations in the animals' requirements. Over and above all this, biotic factors are apparently ignored altogether. It would seem best to study the animals in the field until we know what questions to investigate in the laboratory, rather than blindly accumulating physiological data which may or may not prove to be ecologically significant.

2. Indicator species.

I am prepared to state quite categorically that there is no such thing as a universal indicator species of worm, the presence or absence of which will indicate the degree of pollution by all or any effluent. I venture to suggest that this is true for other taxonomic groups, even more so of entire families, as insects. The foundation for the search for such species, usually in relation to organic pollution, appears to be that the more widely distributed and catholic in its requirements a species is, the more likely it will survive in extreme habitats, free from the competition of more specialized and possibly, thereby, more efficient related organisms. I have been struck by this point in considering the biology of several very common species, not least of which are *Tubifex tubifex* and *Limnodrilus hoffmeisteri*.

May I note at this point that in such abundant and apparently cosmopolitan species there may lie hidden taxonomic criteria that will eventually separate entities presently considered part of a single species; but for the present let us accept the taxonomy as it now stands.

These two tubificids are the first found in any random collection of worms from most parts of the

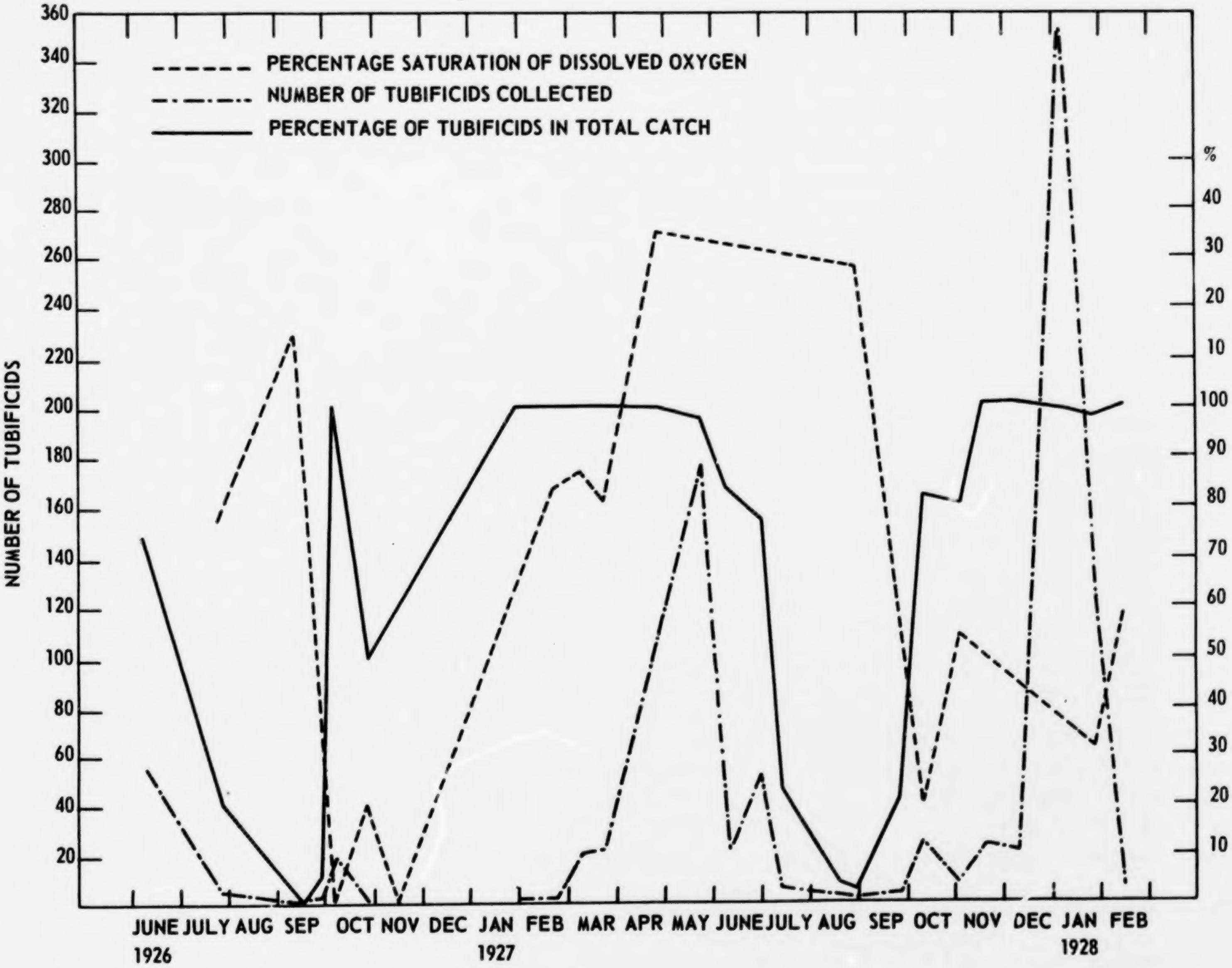

Figure 1. Correlation between the percentage of tubificids in the fauna and the percentage saturation of oxygen in the River Lark.

world. They are found in all sorts of habitat, often together with many other species. They are, therefore, valueless as indicators in the narrow sense. Nonetheless, it is these two species par excellence that reach such astronomic numbers in the absence of predators and competing species, such as erpobdellid leeches and some fishes, when organic pollution reaches such limits as to render the water and the mud anaerobic for prolonged periods. These then would be our polysaprobic species. They are unfortunately not restricted to polluted water, and, furthermore, they are killed by small amounts of metallic poisons. They survive blanketing by inorganic material as long as there is enough organic material to provide (directly or indirectly) a food supply.

It is impossible for an investigator unskilled in biology to identify a handful of worms and report on the status of the water examined. An analysis of the total fauna (including fish if sufficient time and labor is available to collect a series of samples despite shoaling in age classes and if sufficient note is taken of their extreme mobility in assessing the results of analyzing such samples), preferably the bottom fauna of a series of stations with as near uniform a substratum as possible, may be of great diagnostic value if as many species as possible are identified. I will now outline the sort of pattern that may emerge in relation to worms when we have surveyed enough waters in which the sites and nature of effluents are known.

3. Analyses of worm populations in polluted rivers.

There are of course many examples of the abundance of tubificids below sources of organic pollution. I shall take the study of the River Lark (Butcher et al., 1930) as my example, as this is about the first of such studies of a British river. Figure 1 shows the correlation between the percentage of tubificids in the fauna and the percentage saturation of oxygen. The number of tubificids actually fell when the oxygen level was at a minimum, however. The increase in number of worms after the pollution was attributed not only to favorable conditions and an abundance of food, but also to the absence of predators and competitors i.e. the worms may survive the pollution rather than be favored by it.

Table 4. TUBIFICIDAE FOUND IN VARIOUS RIVERS IN RELATION TO THE DEGREE OF ORGANIC POLLUTION OBSERVED

Status of pollution	Species[c]										
	1	2	3	4	5	6	7	8	9	10	11
Gross[a]	++	++									
	+										
	+										
	+										
Bad		++		+							
					+						
	+	+	+								
	+++	++	+								
	++	++	+								
	++	+	+	+							
	++	+			++						
Recovery or slight	+	+++	+		+					+	
	+				+				+		
		+		+	+						+
	+	+	+	+							
	+	+	+		+						
			+	+							
	+	+	+	+	+						
		++	++	++	+	+	+			+	
		+++		++	+			+			
[b]	++	+							+		
[b]	+	++							+		
		+							+		
Clean (Actual numbers given)	1	4			1		3	1·	15		

[a] There is a very large number of streams in this category in which species 1 and 2 are very abundant.

[b] There are several examples of these patterns.

[c] 1. *Tubifex tubifex*, 2. *L. hoffmeisteri*, 3. *L. udekemianus*,
4. *E. hammoniensis*, 5. *P. barbata*, 6. *T. ignota*, 7. *E. moldaviensis*,
8. *A. pluriseta*, 9. *R. coccineus*, 10. *E. bavaricus*, 11. *P. velutinus*.

Table 4 gives enough of a rough indication of the relative abundance of tubificids in a series of organically polluted rivers to give a general idea of the sort of pattern which may emerge from detailed research, although I realize a certain degree of circular argument is involved here. Parallel investigations on the rest of the fauna and the chemical conditions of these rivers were sometimes available to support the bald assertion regarding the degree of pollution, but the data available on the worms are not sufficiently detailed to warrant their inclusion. I repeat that this is intended to give a superficial view of what we might expect from a future research program.

In two surveys there is a little more information available. The first example (for which I am indebted to Dr. Hynes) shows how oligochaetes may become abundant below a source of organic pollution. The figures refer mostly to *Tubifex tubifex* and to the Lumbriculidae, *Lumbriculus variegatus* and *Stylodrilus heringianus* (Table 5). The suspended matter in the polluting material may well make the river a more suitable habitat for Lumbriculidae (which are sparce above the effluent), but the effects of the local deoxygenation seem to vary considerably from year to year, possibly due to variation in the degree of pollution. The point of entry of the polluting material is obvious in both parts of the table, and there may be a correlation between the severity of the pollution, the abundance of the Tubificidae and (negatively) the abundance of the Lumbriculidae.

The second example concerns another large British river which has been under investigation for some years and has included six surveys. The sites studied were all sand-gravel banks which usually support few species of tubificid other than *Rhyacodrilus coccineus*. The results of the most recent survey (Table 6) are given in detail, and the effects of the major effluent may be seen. The river is now much cleaner than in previous years (when the BOD reached 25 ppm in midsummer as opposed to 4 to 6 ppm now), and this is illustrated in Table 7 where the results of all surveys are given for the four stations below the effluent.

From these and other surveys we may attempt the following tentative summary:

(1) *Tubifex tubifex* and *Limnodrilus hoffmeisteri*.

These two species are the most resistant to organic and inert mineral pollution in Britain. They occur together in varying proportions in the absence of other species. They may reach astronomic numbers in the absence of predators, especially erpobdellid leeches. When these leeches are present, the standing crop of worms may be low but the productivity high.

(2) *Euilyodrilus hammoniensis*, *Psammoryctes barbata*, and *Limnodrilus udekemianus*.

These species occur in organically polluted water where the number of worms is still high, but they are apparently a little less resistant than the above (vide Berg, Jonasson, and Ockelmann, 1962). *P. barbata* may be very common in grossly polluted streams where the oxygen level is still high owing to rapid, very turbulent flow. (Only the third of these species has been found in the United States to date).

(3) *Rhyacodrilus coccineus* appears to prefer sandy gravel to mud, seems to be able to withstand slight pollution, but is more sensitive than any of the above.

(4) Many other species seem to occur only in clean streams.

The pattern seems to be similar on other continents from which I have received samples, but some other species may enter the pattern such as *Branchiura sowerbyi* (Allanson, 1961; Vaas and Sachlan, 1955).

We have even less information about other families of Oligochaeta. *Nais elinguis* (Naididae) has frequently been found in large numbers in organically

Table 5. THE SMALL OLIGOCHAETA OF A POLLUTED RIVER (data from Dr. H.B.N. Hynes)[a]

	Annual survey No.	Stations												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Tubificidae (mostly *T. tubifex*)	1			1	14	2		2				1	-	-
	2				2		1							-
	3				44	4		2				-		-
	4				148	1	11			1		-		
	5				22							-		
	6			1	127		1		2			-		
	7				1820							-		
Lumbriculidae *(S. heringianus* *L. variegatus)*	1					4	2	24	2	5	1	1	-	-
	2						3			2	2		1	-
	3			1		2	1	4				-		-
	4		1		1				1	1	4	-	1	2
	5				4	10				4	3	-		
	6				13	6	2	1	7	5	2	-		
	7											-		

[a] Tributary introducing organic matter enters the river between stations 3 and 4.

Table 6. THE SMALL OLIGOCHAETES OF A POLLUTED RIVER

Species	Stations[a]												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Tubifex tubifex	5	1	c	1		c	c	c	6	c	5	c	4
T. tempeltoni							c						
Limnodrilus hoffmeisteri	14	2	1	21	c	15	6	1	10	32	42	45	17
L. udekemianus	c					1		c			c	c	c
Euilyodrilus hammoniensis	c		c	c				c	6	1	c	c	2
E. moldaviensis				1									
E. bavaricus	c												
Rhyacodrilus coccineus	7	4		2	8	8	1	4	c		c	3	
Aulodrilus pluriseta	1												c
Bothrioneurum vejdovskyanum										3			9
Psammoryctes barbata			1	c	1	6	4			1	3	2	2
Peloscolex ferox	c												
Nais elinguis	4	8	b	12		3	b		6	1			
Ophidonais serpentina	1												
Stylodrilus heringianus	8	28		1	6	1	2						
Enchytraeidae indet.			47	6	35				2	4			

[a] 1 - above most pollution.
2,3 - below organically polluted tributary.
4 - below an effluent (BOD 50-100 ppm, flow 0.1 mgd).
5,6 - above main sewer.
7 - below main sewer (BOD 12, flow 12 mgd).
8 - Intermittently stagnant cut.
9-13 - mostly recovery, with small sewage works effluent.

b - very abundant

c - present in previous surveys

Table 7. TUBIFICIDAE OBSERVED AT FOUR STATIONS BELOW THE MAIN SEWAGE WORKS EFFLUENT ON A POLLUTED RIVER (as in Table 6) OVER SIX SUCCESSIVE SURVEYS

Species		Station 7						Station 8					Station 9						Station 10					
	Survey	1	2	3	4	5	6	1	2	3	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Tubifex tubifex		++	++	++	++	2	0	++	++	++	1	0	++	++	++	++	9	6	++	++	1	+	1	0
T. templetoni						1																		
Limnodrilus hoffmeisteri		++		++	++	16	6	++	++	++	3	1	++	++	++	++	5	10		++	4	+	5	32
L. udekemianus										+	1													
Euilyodrilus hammoniensis				+		8			+	+	4		+	+	+		6	6			6	+		1
Bothrioneurum vejdovskyanum																								3
Rhyacodrilus coccineus					+	1	1					4				+								
Psammoryctes barbata							4																	1
Total numbers of all worms in sample.		A	A	A	16	A	40	A	A	1	16	5	A	A	A	40	42	45	A	A	11	16	6	60

A-Very abundant, impossible to estimate by the technique used.

polluted streams (especially where *Cladophora* is abundant) and *Stylodrilus heringianus* (Lumbriculidae) seems to tolerate a certain amount of pollution.

Conclusions.

1. We know very little about the biology of aquatic oligochaetes.
2. What little we do know indicates that detailed quantitative surveys of the species occurring below known sources of organic pollution will enable us to use tubificids, in particular, in the detection and assessment of pollution in other rivers.
3. A marked absence of oligochaetes and other soft-bodied animals in apparently suitable habitats may be useful in the detection of effluents containing poisonous metal ions.

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DISCUSSION

The discussion centered around the following headings: The Winogradsky column, Protozoa and toxicity, pollution indicators, and definitions of pollution. In addition to the chairman and speakers, persons taking part in the discussion included B. R. Allanson, T. W. Beak, R. J. Benoit, W. D. Burbank, P. Doudoroff, W.F. Hahnert, T. E. Maloney, W. Ohle, R. Patrick, W. E. Royce, R. E. Warner, C. G. Wilber, and C. B. Wurtz.

The Winogradsky column compares favorably with conditions along the shore but not at the bottom of a pond. The photosynthetic bacteria in it use scavenger light. On the lower side of a lilly leaf they use what light is left. A fluorescent lamp will not cause the bacteria to develop.

Protozoa are relatively insensitive to toxicants, an example being nerve gases. Protozoa are not related to organs of higher animals and man. Surprise was expressed that Protozoa are insensitive to nerve gases since *Tetrahymena* is sensitive to them, the response test utilizing movement. If pure cultures are used *Tetrahymena* is found to be sensitive to botulinus toxin. Ciliates are sensitive to colinesterase. Critical tests on many of these have not yet been published.

As to indicator organisms for polluted water, the reproducibility of samples is a problem. The number of species of Protozoa that might be identified is endless, but of the common forms, there are about 55-60 species. There appears to be no fixed community of Protozoa at the genus level. At the species level, there is, for the weed species. It is inadvisable to look at only one segment of the community. Great consistency has been found in specific places but chance may play an important role here.

It was suggested that far too much reliance is placed on the saprobic system. What is the mesosaprobic zone in terms of chemistry? High or low BOD or COD is not satisfactory. Velocity variations are important because the rate of flow causes changes in the fauna. The polluted stream is a complex system; therefore, there are many exceptions. Large numbers of organisms occur at the point where predators are removed. Some organisms are associated with warm water discharge; some are much larger in size in the warm water. The amount of oxygen present may or may not be an important factor. A form such as *Tubifex tubifex* is one that survives in the polluted area but requires a muddy substrate to do so. Possibly we need to get away from the idea that correlations on a field basis can be developed. A fundamental basis is needed for correlations. We are forced into a corner at the moment as to interpretation of results.

The meaning and value of the term pollution was discussed. Pollution is essentially an increase in the amount of food available and toxicity is a separate consideration; but there was objection to this as metals, etc., are needed. Adjectives may be used, such as organic pollution and toxic pollution. If we define pollution in terms of our own interests we get many different ones. In a sense, the term pollution is out of date because we lump so much under it. It may be useful only for lay groups. Several terms have become meaningless except for laymen and non-technical persons, but since they do have this use, a word such as "pollution" will have to be continued.

ENVIRONMENTAL REQUIREMENTS OF MARINE INVERTEBRATES

M.R. Carriker, Chairman*

BIOASSAYS OF PULP MILL WASTES WITH OYSTERS

Charles E. Woelke †

INTRODUCTION

Between 1924 and 1928 sulfite pulp mills began discharging wastes into the waters of three of the four principal oyster producing areas of Washington State. Within a few years laboratory research was begun to measure the effects of pulp mill wastes on oysters. Research of Hopkins, Galtsoff, and McMillin (1931) demonstrated the toxicity of calcium-base sulfite waste liquor (SWL) to oysters at concentrations between 500 and 10,000 ppm (based on 10% total solids). Kincaid and Benson observed 18.7 percent mortality of Olympia oysters *(Ostrea lurida)* in their controls and 34.4 percent mortality in 100 ppm after 132 days (Gunter and McKee, 1960). Odlaug (1949) found complete cessation of pumping by Olympia oysters after 15 days in 100 ppm of fresh calcium-base sulfite waste liquor. Liquor which had been stabilized for several months had less effect on the oysters. McKernan, Tartar, and Tollefson (1949), in spite of high control mortalities, concluded from their 575 day bioassay of calcium-base sulfite waste liquor with Olympia oysters that: "Statistical analysis of these results indicated an undeniable correlation between the mortality of the oysters and concentration of SWL even at these low concentrations; concentrations at least as low as 13.0 ppm are inimical to continued Olympia oyster culture." The findings of these researchers clearly established the toxicity of sulfite waste liquor concentrations in excess of 100 ppm. In the mid 1950's excessive mortalities (Woelke, 1956), reduced growth (Woelke, 1958), poor condition (Westley, 1959b), and reproductive anomalies (Westley, 1959a) of Olympia oysters were recorded.

Between 1956 and 1958 both the Washington Department of Fisheries and the Washington Pollution Control Commission conducted extensive water sampling programs in all oyster growing areas in Washington. Sulfite waste liquor concentrations based on 10 percent total solids, as measured by the Pearl-Benson (1940) test, ranged from 0 to 143 ppm over the oyster beds. The Pearl-Benson indices varied within areas, between areas, and seasonally. Most sample values over oyster beds were less than 20 ppm, and the trip averages were usually less than 10 ppm (Westley, 1960; Wash. Poll. Control Comm., 1957a, 1957b, 1958).

METHODS

Since the sulfite waste liquor concentrations (as measured by the Pearl-Benson test) in oyster growing areas were generally less than those studied by earlier researchers, a new bioassay program was initiated in 1956. In brief, this involved: further statistical analysis of the results of McKernan et al, (1949); placing floating live boxes at various distances from a source of pulp mill waste to bioassay the existing field conditions relative to growth, mortality, and fatness of Olympia oysters; a 3-year laboratory bioassay with Olympia oysters of the concentrations of sulfite waste liquor found over oyster beds; laboratory bioassays of low concentrations of sulfite waste liquor with larvae of Olympia and Pacific *(Crassostrea gigas)* oysters; bioassay of sulfite waste liquor with Olympia and Pacific oysters in a lagoon; and 48-hour bioassays with fertilized Pacific oyster eggs exposed to water from areas receiving pulp mill wastes. By following this approach, a bridge of biological responses was established from the original oyster populations, to field live boxes, to laboratory bioassays, to lagoon bioassays, and back to bioassays with oyster larval development in water samples from oystering areas with and without pulp mills.

RESULTS

Probit Analysis of McKernan et al. (1949) Data

Since high control mortalities (52% and 53%) raised considerable doubts as to the validity of the results reported in 1949 by McKernan et al., a more careful statistical analysis was made of their data by Junge (1956). Junge (op. cit., page 6) stated that: "Standard statistical procedures applied to these data permit the calculation of mortalities due to the sulfite waste liquor alone for any concentration within the effective range. Various mathematical considerations of the data indicate that the experimental work was well controlled and that the calculated regression line given in Figure 1, gives a very satisfactory representation of the results of the experiment. The 95 percent confidence interval on the expected mortalities is also plotted in Figure 1, and the mortality rates are calculated for the lower concentrations in Table 2."

In Junge's analysis he used "Abbott's formula" to compute an adjusted mortality which could be attributed to sulfite waste liquor alone. He then plotted the adjusted mortality against the logarithm of concentration on probability paper. Using the procedures outlined by Finney (1952), a probit regression line was fitted to the data (Figure 1). He then utilized the formula for the regression line to compute the values in Table 1. From Junge's analysis,

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I concluded that the concentrations of pulp mill waste present in Washington waters could be having an effect on the Olympia oyster mortalities.

FLOATING LIVE BOXES

In December 1956, floating live boxes containing 2-, 3-, and 4-year-old Olympia oysters, were located at various distances from the waste outfall of an ammonia-base sulfite pulp mill located at Shelton, Washington. Oysters collected from the same oyster bed were cleaned of exterior material and their size determined by volume displacement before being put into the live boxes. Mortality and size of the oysters were determined at least once a month. Water samples collected adjacent to the live boxes were analyzed for sulfite waste liquor and salinity. Fatness of the 4-year-old oysters, as measured by the relationship between dried meats and volume of shell cavity, was determined on February 1, 1958.

Since the pulp mill ceased operations in August 1957, the 13 months of data summarized in Figure 2 reflect oyster responses for 8 months during which the pulp mill was in operation, followed by 5 months during which the mill was not operating. Data from the five live boxes located 0.75, 3, 10.5, 13, and over 50 miles from the pulp mill showed no apparent pattern of growth differences. Condition factor was poorest closest to the pulp mill, with no particular pattern in locations farther from the mill. Mortality was highest closest to the mill, with no particular

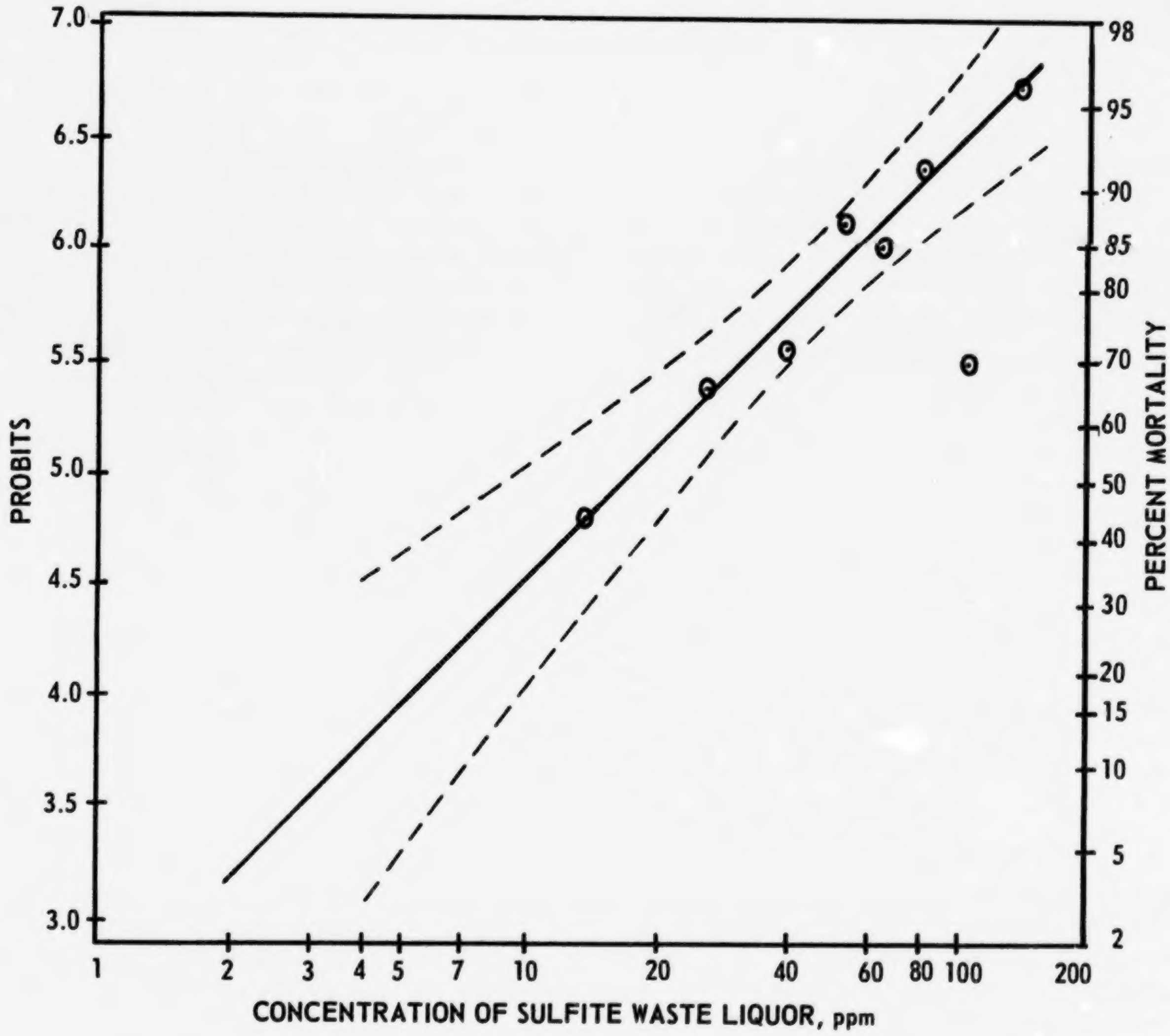

Figure 1. Junge's Probit analysis graph of McKernan, et al's 49A data.

Table 1. CALCULATED MORTALITY AND 95 PERCENT CONFIDENCE INTERVAL FOR LOW CONCENTRATIONS OF SULFITE WASTE LIQUOR

Concen. S.W.L. (ppm)	Calculated mortality (percent)	95 percent confidence interval
4	11.7	2.7 to 32.3
10	33.6	18 to 52.6
13	41.9	26.5 to 58.7
20	56.3	43.5 to 68.4
30	69.0	59.9 to 77.1

pattern farther from the mill. The Pearl-Benson indices averaged over 7 ppm at the live box nearest the mill, and averages were less than 4 ppm at the other boxes. Since the Pearl-Benson test measures color producing materials other than sulfite waste liquor (commonly referred to as "background"), it is questionable whether more than a trace of pulp mill waste was present except at the live box closest to the pulp mill.

These data suggest that the concentration of pulp mill wastes at the live box closest to the mill adversely affected the mortality rate and fatness of the Olympia oysters but probably did not affect the growth.

LONG-TERM BIOASSAY

In December 1958, a 3-year bioassay of ammonia-base sulfite waste liquor was begun. The bioassay was designed to measure the effects of 2, 4, 8, 16, 32, 64, and 128 ppm of sulfite waste liquor (based on 10 percent total solids) on the mortality, growth, fatness, reproduction, feeding, and histology of Olympia oysters.

The bioassay was started with an initial population of 5,400 oysters; each concentration and the two controls included 200 one-year, 200 two-year, and 200 three-year-old oysters. The physical facilities, methods for supplying a continuous flow of the waste, and other details of the study have been previously reported (Woelke 1960a).

The bioassay was terminated in January 1962, and the data are being analyzed at the present time; however, during the course of the study, preliminary analysis of the mortality data was made. In Figure 3, mortality data from the 1956 and 1957 year-classes of Olympia oysters exposed to sulfite waste liquor for 575 days are analyzed in the same manner used by Junge (op. cit.) and compared with Junge's analysis of the data of McKernan et al. The regression lines from the 1956 and 1957 year-classes are nearly identical. These lines, however, are at a lower level and have less slope than that of the data of McKernan et al. These differences indicate a lower level of toxicity in our long-range bioassay than those reported in 1949. While the possible reasons for these differences are of concern, the main point of interest lies in the apparent confirmation of conclusions by McKernan et al, that low concentrations of sulfite waste liquor have adverse effects upon survival of Olympia oysters.

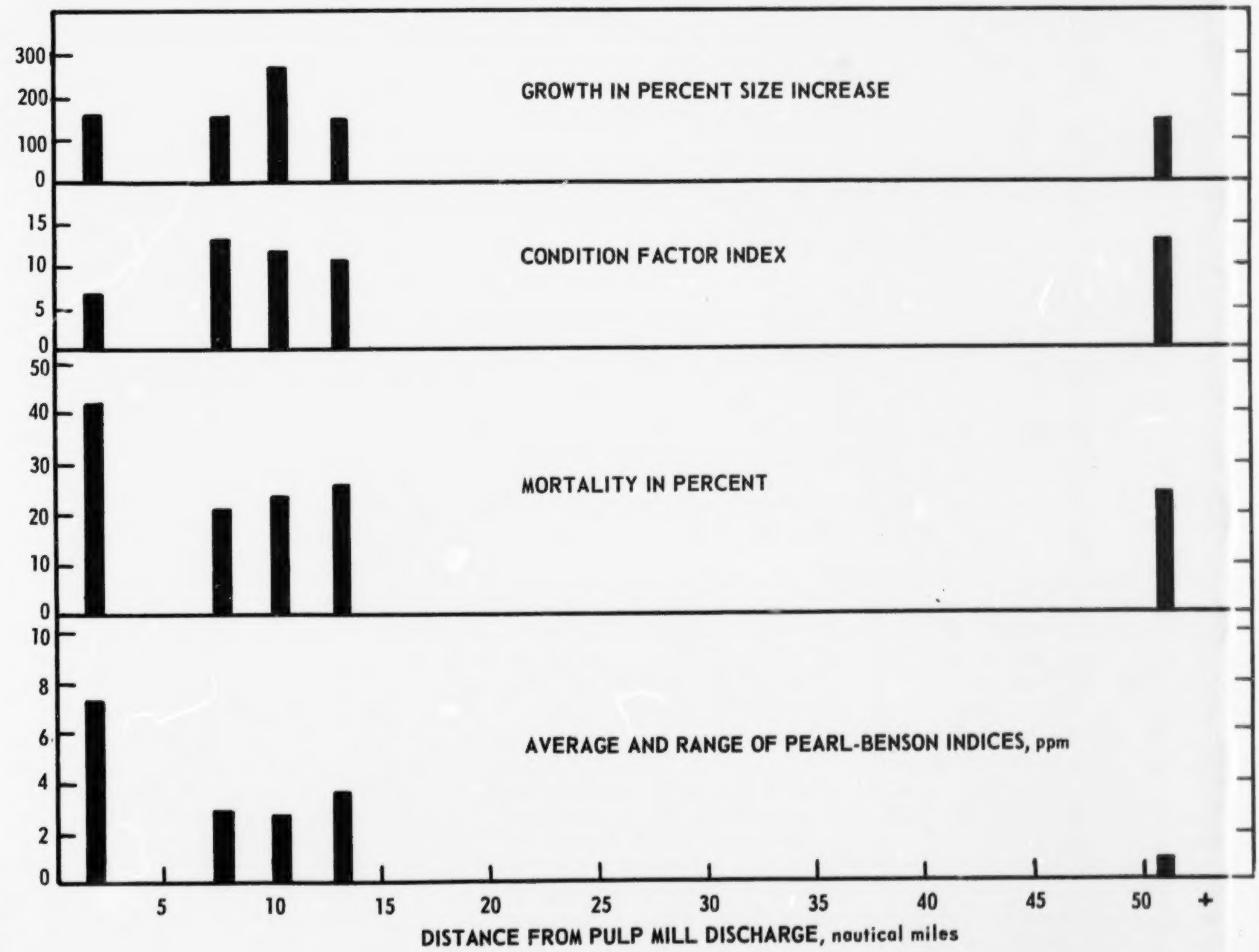

Figure 2. Results from 5 stations of floating live box study.

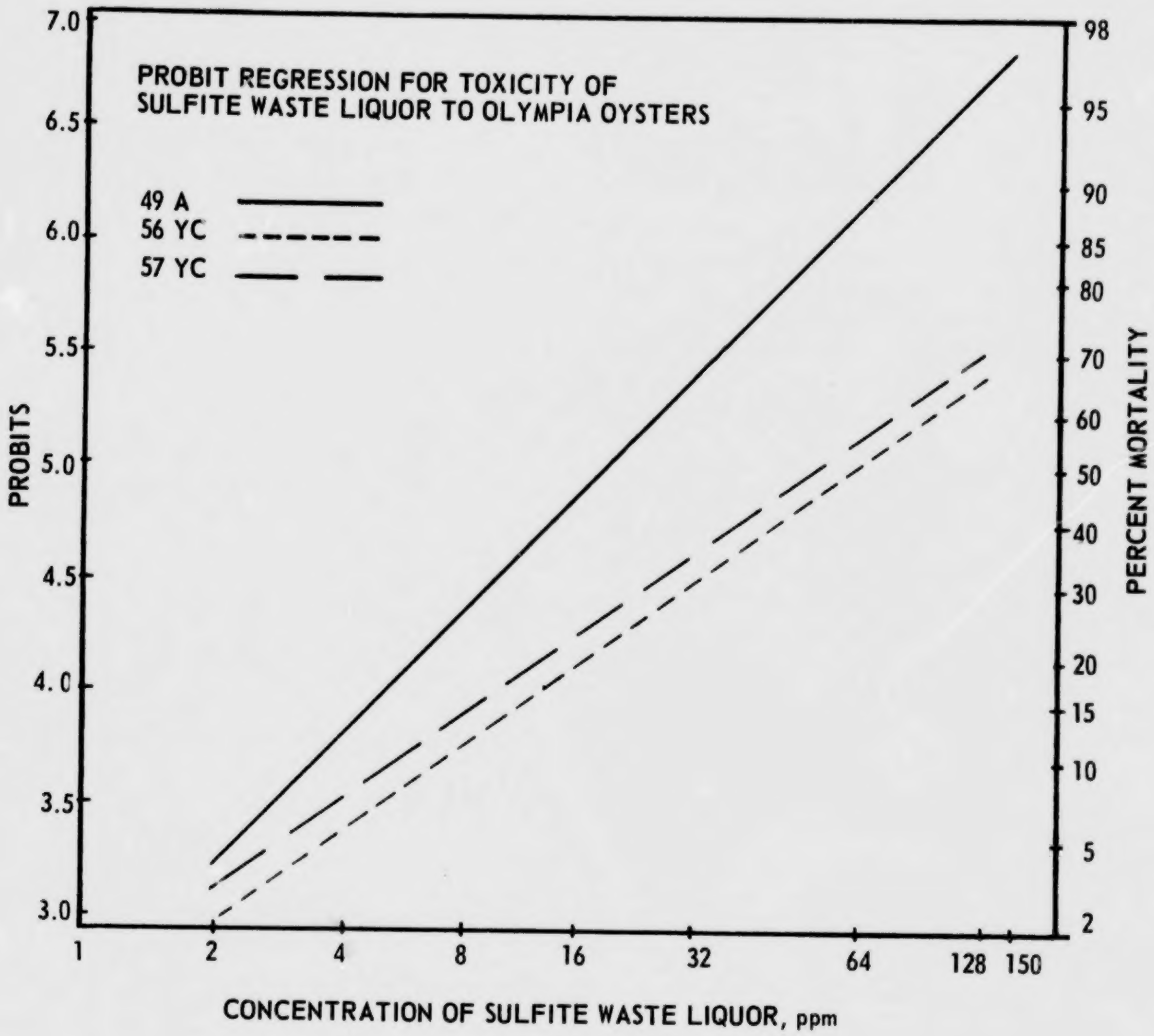

Figure 3. Probit regression for toxicity of SWL to Olympia oysters.

Table 2. SUMMARY OF *OSTREA LURIDA* LARVAL PRODUCTION PER 600 OYSTERS BY CONCENTRATION OF SULFITE WASTE LIQUOR.* JUNE 8 THRU AUGUST 7, 1959

Tray	Larval counts			Percent abnormal	Calculated larval production						Larval size	
	Total	Normal	Abnormal		Number of tows	Larvae in tows	Per tow	Total produced (thousands)	Total larvae per oyster	Normal larvae per oyster	No.	Average size
Header box	20	20	0	0	174	1,313	8	65.2			19	165.0
Control (01)	7084	7068	16	0.22	169	442,750	2620	21,844.9	36,408	36,328	367	157.8
Control (02)	9069	9055	14	0.15	169	566,813	3354	29,151.2	48,585	48,512	361	158.6
2 ppm	5923	5912	11	0.19	166	370,188	2230	19,242.4	32,071	32,010	360	154.9
4 ppm	5793	5732	61	1.05	167	362,063	2168	18,816.4	31,367	31,038	376	153.3
8 ppm	5893	5349	544	9.24	170	368,313	2167	18,791.3	31,319	28,429	364	150.9
16 ppm	6117	2112	4005	65.46	172	382,313	2223	19,180.6	31,634	10,927	345	139.2
32 ppm	901	25	876	97.22	168	56,313	389	2,895.6	4,826	134	23	161.2
64 ppm	173	13	160	92.48	171	10,813	63	546.3	910	58	13	159.2
128 ppm	12	12	0	0	171	750	4	37.9	63	63	12	164.1

* In view of the small numbers of larvae produced from the header box, no correction has been made for "wild larvae" entering the sea water supply.

OLYMPIA OYSTER LARVAL BIOASSAYS

In view of the anomalies observed in reproduction of Olympia oysters in the field in 1953, 1954, 1955, and 1956, particular attention was given to the possible effects of sulfite waste liquor on the reproduction of Olympia oysters in the laboratory studies. Woelke (1960a) has described the effects of various concentrations of sulfite waste liquor on the spawning, swarming, and setting of Olympia oysters. Most of these data were collected in conjunction with the 3-year bioassay of sulfite waste liquor with Olympia oysters.

In Figure 4 the spawning and brood development of Olympia oysters are summarized. While spawning occurred at all concentrations bioassayed, embryonic development from eggs to shelled larvae did not take place in concentrations above 16 ppm. At 16 ppm only limited development occurred. The development at 2, 4, and 8 ppm was less than in controls. Larval production, as determined by sampling water discharge in the 3-year bioassay, is summarized in Table 2. Very few wild larvae entered the sea water system to affect the numbers found in the sampling. In the two controls sampled, percent "abnormal larvae", i.e., larvae that are not fully shelled (Figure 5), was lower. Numbers of larvae and average size of larvae in the two control samples were superior to those at (of) any of the concentrations bioassayed. At 8 ppm there was marked increase in abnormal

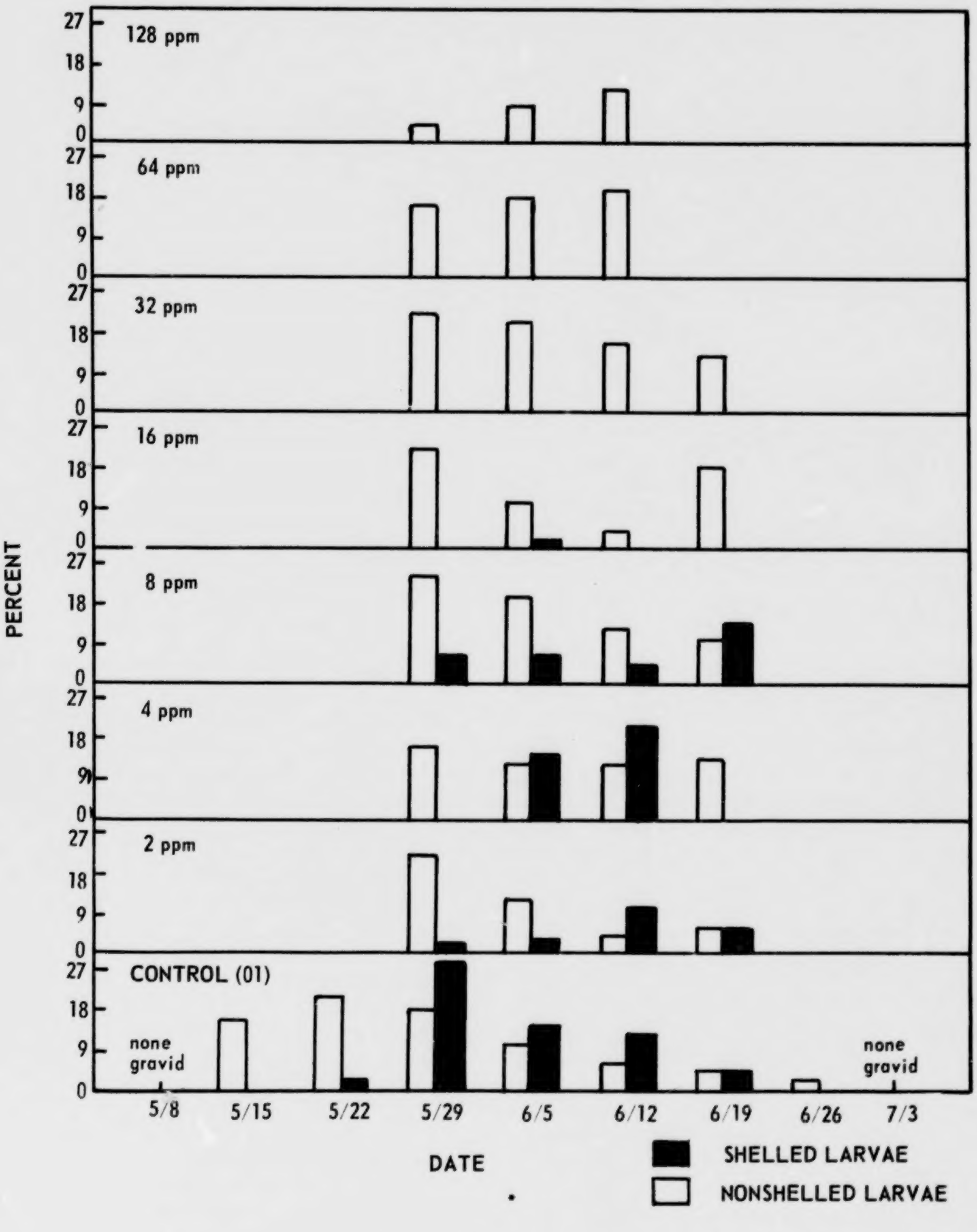

Figure 4. Olympia oyster spawning bioassay.

larvae, decrease in numbers of larvae, and decrease in size of larvae collected. At 16 ppm over 50 percent of the larvae were abnormal, numbers of normal larvae were reduced by over 60 percent and size of the larvae produced was reduced by over 12 percent. Above 16 ppm no normal larvae were produced.

Larvae spawned in the controls and in 2, 4, 8, and 16 ppm of sulfite waste liquor were cultured in the laboratory. Larvae from all except 16 ppm were successfully reared to juvenile oysters. Repeated efforts to raise larvae spawned in 16, as well as 32, 64, and 128 ppm, were not successful.

Failure to rear larvae spawned in 16 ppm led to the conclusion that an increase in percent abnormal larvae represents an adverse effect on oyster reproduction. It was further concluded that, under the conditions of these bioassays, fresh ammonia-base sulfite waste liquor will interrupt the life cycle of the Olympia oyster at 16 ppm. Concentrations of 2, 4, and 8 ppm affect the reproductive cycle; however, these effects were not necessarily adverse.

PACIFIC OYSTER LARVAL BIOASSAYS

While the Pacific oyster is the most valuable shellfish resource of Washington State, a very few reports on its response to sulfite waste liquor have been published. In 1958, laboratory bioassays of sulfite waste liquor with 48-hour larval development of fertilized Pacific oyster eggs were started. The results of these bioassays were reported by Woelke (1960b). The report was based on the results of 338 larval cultures. In these studies about 15,000 fertilized Pacific oyster eggs were added to 1-liter beakers containing concentrations of the waste to be bioassayed. After 48 hours the cultures were sampled and the percent of abnormal larvae determined (Figure 2).

The results of these studies are summarized in Figure 7. Concentrations between 1 and 6 ppm caused an increase in the percent of abnormal larvae above the level of the controls. Between 6 and 12 ppm, over 20 percent of the larvae were abnormal; from 12 to 18 ppm, nearly 60 percent were abnormal; and above these levels, over 90 percent were abnormal.

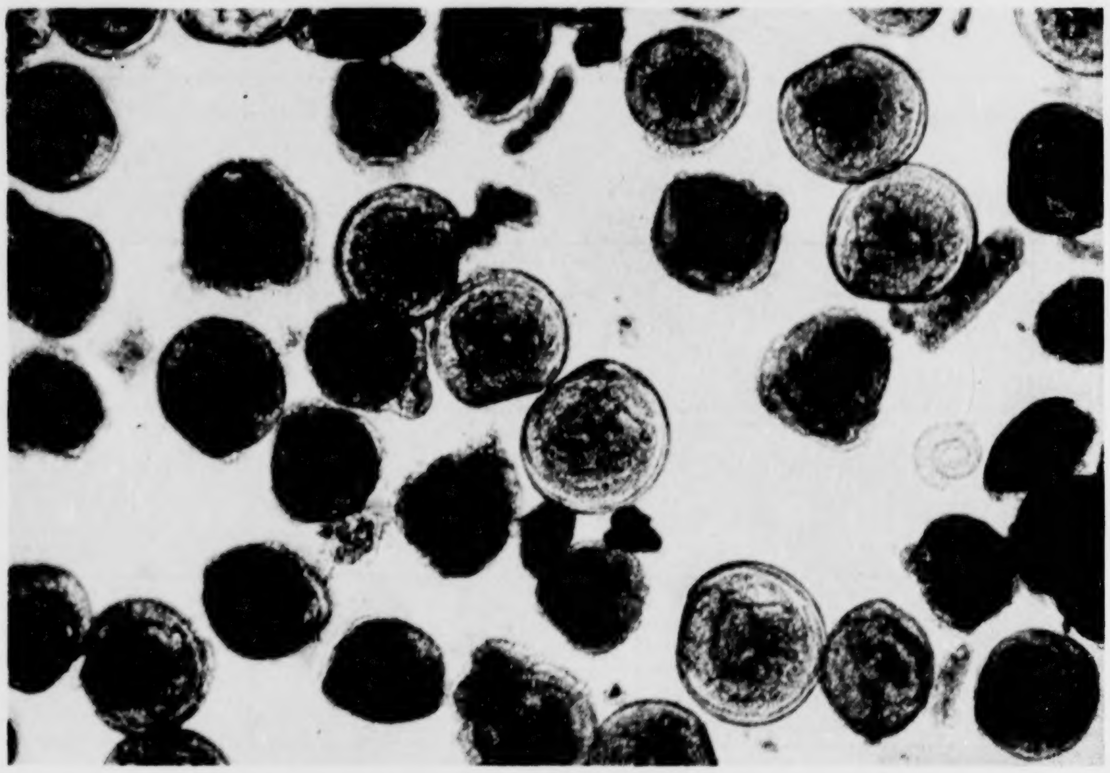

Figure 5. Normal and abnormal Olympia oyster larvae.

Table 3 compares development of eggs fertilized in sulfite waste liquor with eggs which were fertilized before introduction into the waste. In this experiment the percent abnormal larvae in 3 ppm was 20 percent more than the controls.

From these experiments it was concluded that: fresh sulfite waste liquor affected 48-hour development of fertilized Pacific oyster eggs at concentrations of 2 ppm and greater; above 18 ppm, fresh sulfite waste liquor caused nearly 100 percent of the developing 48-hour larvae to be abnormal; in some experiments over 90 percent of the larvae were abnormal at 12 ppm. Below 13 ppm, 48-hour Pacific oyster larvae developing from eggs fertilized in sulfite waste liquor had higher incidence of abnormality than those introduced into the waste 1 hour after fertilization.

LAGOON STUDIES

In 1958, 1959, and 1960 experiments measuring chemical changes of sulfite waste liquor in sea water were conducted in a lagoon near the Point

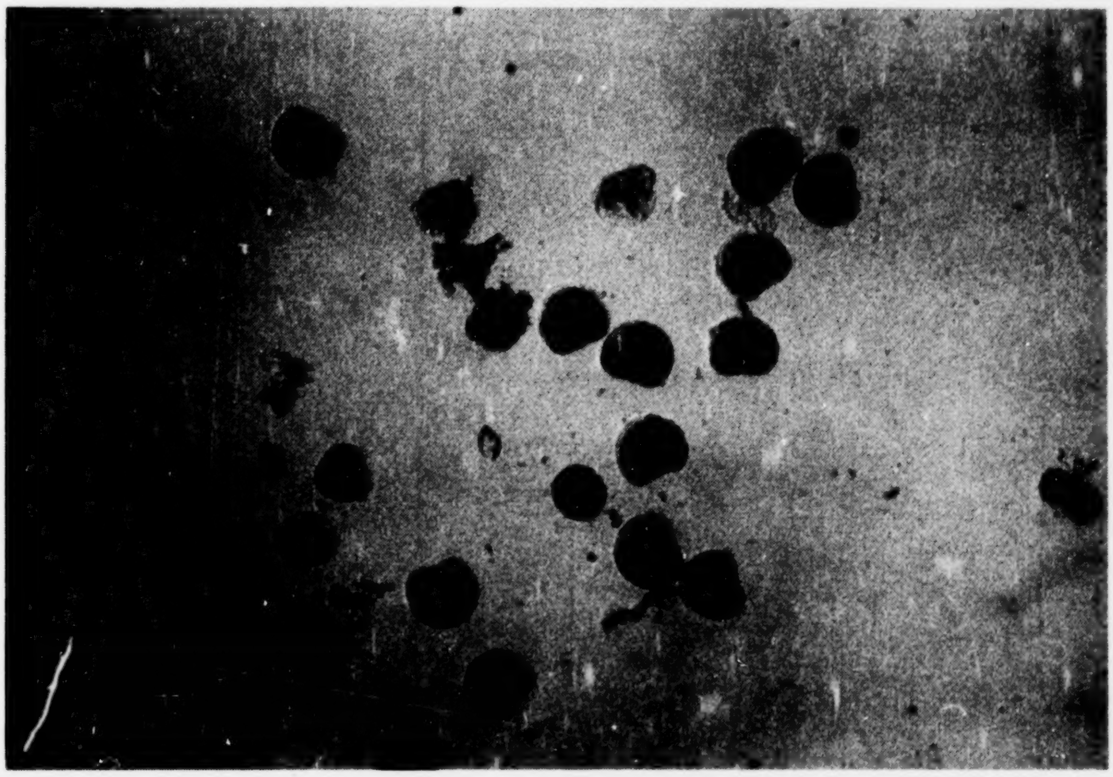

Figure 6. Normal and abnormal Pacific oyster larvae.

Table 3. PERCENTAGE OF ABNORMAL LARVAE FROM EGGS FERTILIZED IN SULFITE WASTE LIQUOR AND FROM EMBRYOS 1 HOUR OLD WHEN EXPOSED TO WASTE

Fertilized in S.W.L.		1-hour embryos	
Pearl-Benson Index	Percent abnormal	Pearl-Benson Index	Percent abnormal
0	6.3	0	4.3
1	10.8		
		2	6.2
3	26.8	3	4.3
6	23.7	6	4.7
13	99.2		
		14	99.4
30	100.0	31	100.0

Whitney, Washington, Shellfish Laboratory. A 2-1/2-acre portion of the lagoon is diked, and ingress and egress of water is controlled through two 30-inch-diameter pipes. The remainder of the lagoon is open to the adjacent bay, and daily water exchange akes place. The pipes of the diked portion of the agoon were closed and sulfite waste liquor added until the desired Pearl-Benson index was achieved.

In one experiment 13 ppm was maintained for over a year. Additional waste was added every 7 to 10 days to maintain this Pearl-Benson index. Experimental populations of both Pacific and Olympia oysters were placed on trays inside the dike, and controls were placed in similar trays outside the dike. Periodic measurements of growth, mortality, and fatness were made on the oysters.

It is recognized and readily acknowledged that the controls utilized were not all that could be desired, and steps are now under way to bisect the present experimental lagoon with a dike to provide satisfactory controls. Results from the bioassay with the present lagoon indicate adverse effects on Pacific oyster fatness and Olympia oyster mortality at 13 ppm.

Bioassays with Water Samples from Oystering Areas

In 1961 and 1962, bioassays of water collected in the field have been conducted in the laboratory with 48-hour Pacific oyster larval development utilizing the techniques described by Woelke (1961). Surface-water samples were collected by airplane, and fertilized Pacific oyster eggs were added within 3 hours of the time the samples were picked up in the field.

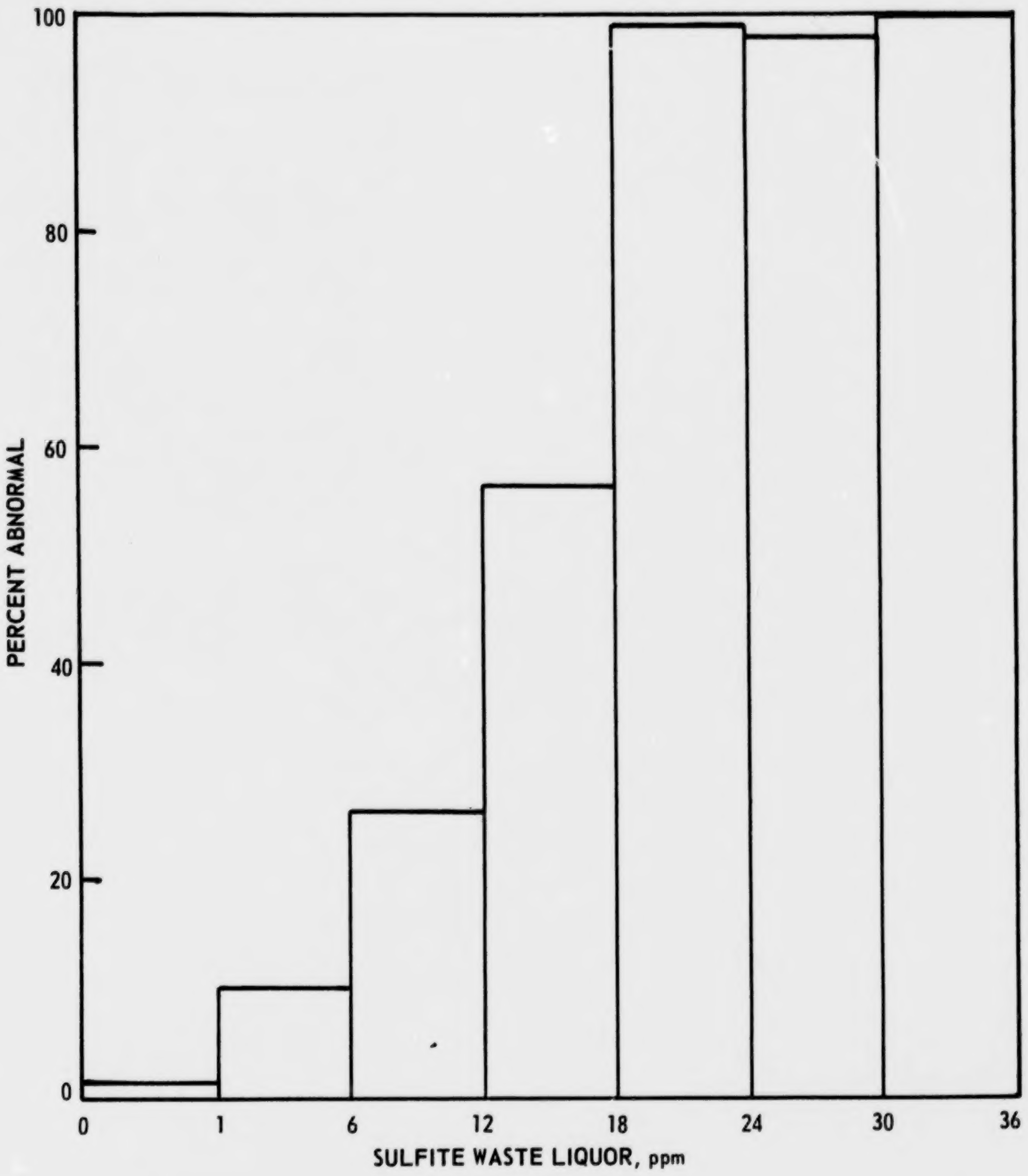

Figure 7. 48-hour development of Pacific oyster larvae in sulfite waste liquor.

To date 10 bioassays of over 180 water samples with more than 500 larval cultures have been conducted. These bioassays included water from oystering areas with and wihout pulp mill wastes.

Results of these bioassays are shown as percent abnormal larvae in Figure 8. The data indicate little difference between laboratory controls and field water samples with a zero Pearl-Benson index. The same is true of Pearl-Benson indices of 1 to 6 ppm. Above 7 ppm the percent abnormals increase substantially, and above 30 ppm nearly 100 percent of the larvae are abnormal.

From these data it is concluded that Pearl-Benson indices above 7 ppm in the natural environment (including "background") have an adverse effect on the 48-hour development of fertilized Pacific oyster eggs.

SUMMARY

The results of the bioassays are briefly summarized in Table 4. The Pearl-Benson index at which adverse effects occur is: an average of 7 ppm on Olympia oysters in floating live boxes; about 16 ppm (15% above control mortalities) on Olympia oysters in the long term laboratory bioassay; between 8 and 16 ppm on Olympia oyster reproduction in the laboratory; 3 ppm on Pacific oyster eggs fertilized in laboratory mixes of sulfite waste liquor; 13 ppm in lagoon bioassays on Olympia and Pacific oysters; 7 ppm (including "background") on fertilized Pacific oyster eggs in water collected from oyster growing areas.

CONCLUSIONS

Throughout these studies, which began in the field and were followed by laboratory, lagoon, and, finally, field water sample bioassays, the major objective was to determine whether the quantities of sulfite waste liquor in oystering areas was detrimental to oysters. At no point has it been clearly demonstrated that the amounts of sulfite waste liquor present over the oyster beds in Washington waters could be the one and only cause for the death or distress of an oyster or oyster population. On the other hand it is concluded that the amounts of sulfite waste liquor present in some of these waters can create stresses which, if not immediately lethal, may be expected to have a long term adverse effect on oysters.

In comparing the various oyster responses studied, the reproductive stage of the life cycle seems to be the most sensitive to pulp mill wastes. The toxicity of low concentrations of sulfite waste liquor to oysters is clearly indicated. This is especially true when the concentrations bioassayed are considered on the basis of 100 percent total solids, rather than the 10 percent normally used with sulfite waste liquor. When the 100 percent total solids yardstick is used, 5 to 10 ppm is clearly lethal to adults with no safety factor applied, and, on larvae, between 1 and 2 ppm is lethal.

Additional research is needed to demonstrate whether the acute toxicities measured by embryonic development at low concentrations are reflected in less obvious chronic stresses in adults. Future bioassays with water samples from oystering areas shall be conducted in which eggs are fertilized in the samples. Further lagoon bioassays shall be conducted after a satisfactory facility has been constructed.

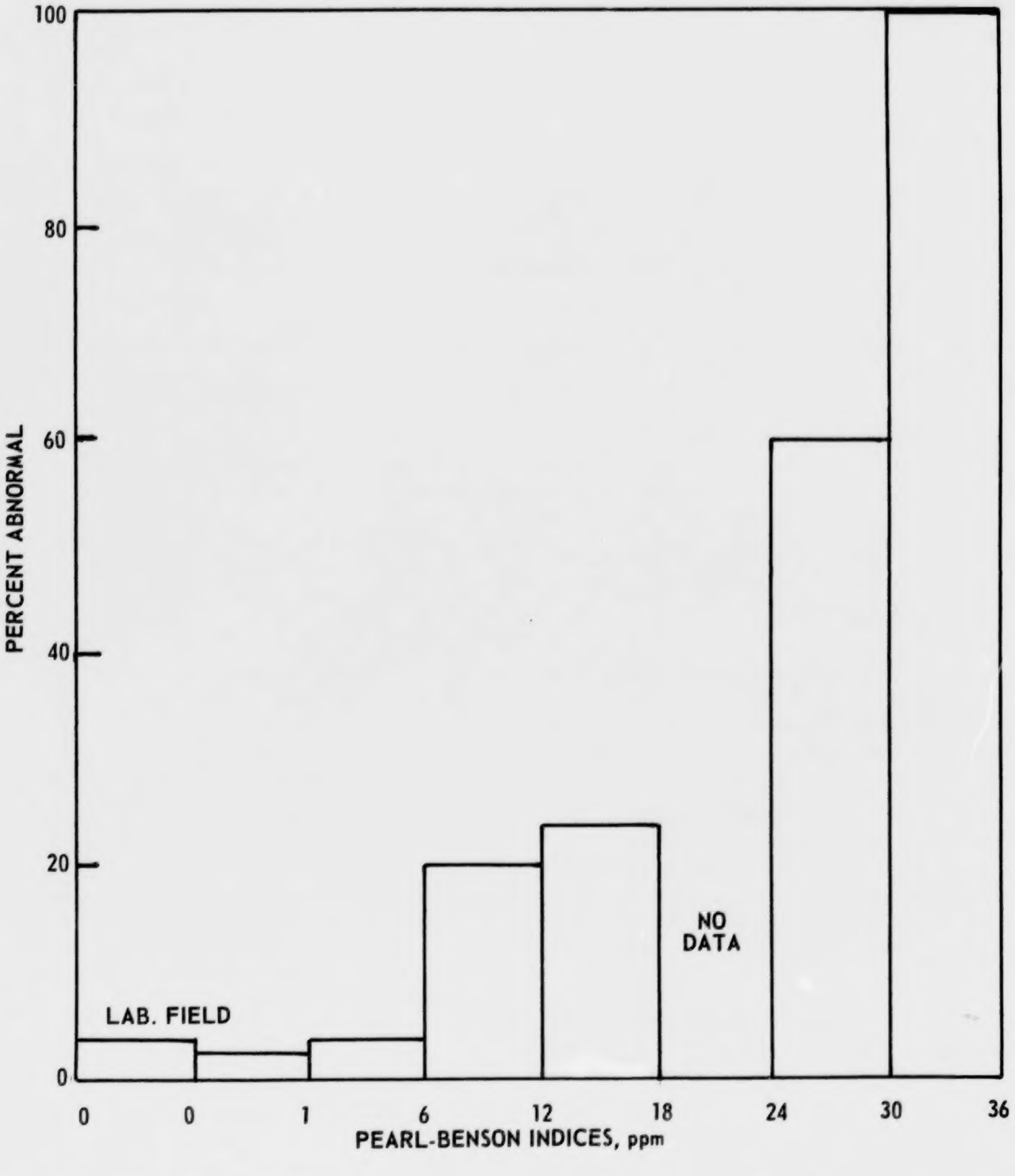

Figure 8. 48-hour development of Pacific oyster larvae in field water.

Table 4. SUMMARY OF RESULTS OF BIOASSAYS OF SULFITE WASTE LIQUOR WITH OLYMPIA AND PACIFIC OYSTERS

Type of bioassay	Type of response measured	P.B.I.* at which adverse effects occurred
Floating live boxes (Olympias)	Mortality and condition index	Average of 7 ppm.
Long range laboratory bioassay (Olympias)	Mortality	About 16 ppm.
Olympia oysters in laboratory	Reproduction	Between 8 and 16 ppm.
Development of Pacific oyster eggs fertilized in sulfite waste liquor	Percent abnormal larvae	3 ppm.
Lagoon bioassays with Olympia and Pacific oysters	Growth and mortality	13 ppm.
Fertilized Pacific oyster egg development in field water	Percent abnormal larvae	7 ppm.

* Pearl-Benson Index

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THE EFFECT OF ENVIRONMENTAL FACTORS ON LARVAL DEVELOPMENT OF CRABS[*]

John D. Costlow, Jr.[†] *and C. G. Bookhout*[‡]

Larvae of the Brachyura, or true crabs, are found in virtually all estuaries and marine environments. In some areas they comprise an important portion of the zooplankton and represent such commercially important species as the blue crab, *Callinectes sapidus* Rathbun, and the stone crab, *Menippe mercenaria* (Say). Development of the Brachyura generally follows a certain pattern; a series of free-swimming stages of the larva, which through successive molts attains an intermediate or transitional form, which in turn metamorphoses to the pre-adult. The majority of life histories that have been published have been based on reconstructions from larvae obtained from the plankton. The usual procedure has been to obtain the first-stage zoeae from a crab that released the larvae in the laboratory, and then attempt to find the sequence of more advanced larvae in the plankton, which appear to match the general characteristics of the hatched first stage. This procedure has resulted in descriptions of life histories that were not complete and occasionally were based on larvae from several different species of crabs rather than on one species. During the past few years techniques have been developed that allow the rearing of crab larvae in the laboratory from eggs, through all larval stages, with a reasonable degree of success (Costlow and Bookhout, 1959, 1960, 1961). In addition to providing positive identification of all larval stages of the species under study, the laboratory rearing also provides large numbers of larvae of known stage of development and age. These can be used for studies on many ecological and physiological aspects of larval development, which previously were impossible or impractical with larvae obtained from the plankton.

Thus far our experiments have been primarily concerned with the effects of three or four environmental factors on larval development. These have included studies on the individual and combined effects of salinity, temperature, diet, and light and how these factors affect duration of individual larval stages; the total time required for development of the crab; the number of larval stages; and the survival of individual stages as well as the overall survival of the crab. Of the 20 species we have successfully reared to date, 4 or 5 species will serve as examples of how salinity and temperature can affect the various phases of larval development of different species.

METHODS

Ovigerous females of *Rhithropanopeus harrisi*, *Panopeus herbsti*, *Sesarma cinereum*, *Hepatus epheliticus*, and *Callinectes sapidus* were brought into the laboratory and maintained separately at 25°C and 30°C in fingerbowls containing filtered sea-water of the salinity in which the larvae were to be reared. With some species the eggs were removed from the pleopods of the female and hatched in plastic compartmented boxes maintained on an Eberbach variable-speed shaker, as described by Costlow and Bookhout (1960 a). At the time of hatching the zoeae were transferred to mass cultures of approximately 500 per bowl and recently hatched *Artemia* nauplii and fertilized *Arbacia* eggs were added immediately. The zoeae were then further subdivided into groups of 10 per bowl and maintained under constant environmental conditions in temperature-controlled culture cabinets. Artificial light for the 14-hour day was provided by MacBeth Examolites, which have a spectral distribution similar to natural daylight. Larvae to be used in the experiments of 20°C were hatched at 25°C and then placed in sea water, the temperature of which was gradually reduced to 20°C. A minimum of 100 larvae were used in each temperature-salinity combination given in Table 1.

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The containers and larvae were examined daily, the zoeae changed to freshly-filtered sea water of the same salinity, and new food was added. At this time the number of molts in each series was recorded and the mortality noted. When the megalops stage was reached the larvae were removed to separate bowls and individual records kept on the time of metamorphosis to the crab.

RATE OF LARVAL DEVELOPMENT

The first crab to be considered is *Rhithropanopeus harrisi*, a Xanthid that successfully inhabits estuarine waters of extremely low salinity. For studies at our laboratory it has been collected from the Newport River in salinities of 0.5 to 25 parts per thousand (ppt). Wurtz and Roback (1955) reported it from rivers emptying into the Gulf of Mexico in salinities that ranged from 0.006 to 22.6 ppt and Ryan (1956) found the adults in portions of the Chesapeake Bay which ranged in salinity from 2.8 to 18.6 ppt. Bousfield (1955) is the only investigator who gives the range of salinity in which the larvae of this species may be found. He collected zoeae from the Miramichi Estuary in Canada in salinities of 4.0 to 23.5 ppt. and found them to be most abundant of 15.0 ppt.

Unlike the famales of some species, ovigerous *R. harrisi* females remain in waters of low salinity. The eggs and larvae of this brackish-water crab have been hatched and reared in the salinity-temperature combinations shown in Table 1. The rate of development of larvae of this species at comparable temperatures is not altered appreciably over the range of salinities used. More recently we have also reared larvae of this species in 35 and 40 ppt and, while development of the crab was observed, preliminary analysis of the data indicated that at 40 ppt there was some slight delay in development. The absence of any appreciable influence of a considerable range of salinities on rates of development of all larval stages of *R. harrisi* is in marked contrast to the effect of salinity on rate of development of larvae of other species that have been reared at our laboratory.

The larvae of a second species of Xanthid crab, *Panopeus herbsti*, have also been reared under different salinity-temperature combinations and are more sensitive to these environmental factors than the larvae of *R. harrisi*. We have collected ovigerous females in the Beaufort area in water of a salinity as high as 33.0 ppt, but the lower limit of the salinity range for the adults is not known. Ryan (1956), studying the distribution of five species of Xanthid crabs in the Chesapeake Bay, found a small number of adult *P. herbsti* in waters ranging in salinity from 13.9 to 19.0 ppt. The present studies have shown that hatching of *P. herbsti* is successful in a salinity range of from 12.5 to 31.1 ppt. The duration of the individual zoeal stages of this species, as well as the time required for complete development to the first crab, are affected by salinity differences (Fig. 1). A delay in the time of molting is evident as early as the first molt for larvae maintained in water at a salinity of 12.5 ppt. The duration of the individual stages of *P. herbsti* was affected by temperature but there was some overlapping of the rates of development of the earlier stages of 25°C. Complete development of the larvae at 30°C required 18 to 28 days. This is approximately half the time observed for comparable development of these same larval stages at 20°C (48 to 52 days).

The larvae of a third species of crab, *Sesarma cinereum*, show even greater effects of salinity on rate of development than the two species previously considered. The adult crab, a small dorso-ventrally depressed crab, which lives under the moist drift line along protected beaches, is found throughout much of the estuarine area adjacent to the Beaufort Laboratory. Pearse (1936), during the summer of 1928, found adult crabs adjacent to waters that varied in salinity from 0.5 to 18.9 ppt. Crabs used in our experiments were collected from areas where the salinity was 26.5 to 35 ppt. While hatching did occur in all salinities used in this experiment (Table 1), the duration of the individual stages and the time required for complete development to the first crab were affected by the salinities used (Figure 2). In the lower salinities, 12.5 and 20.1 ppt, there was a tendency for molting of the individual stages to begin later than at the higher salinities. This is naturally reflected in the time required for complete development (Figure 2). At comparable temperatures there

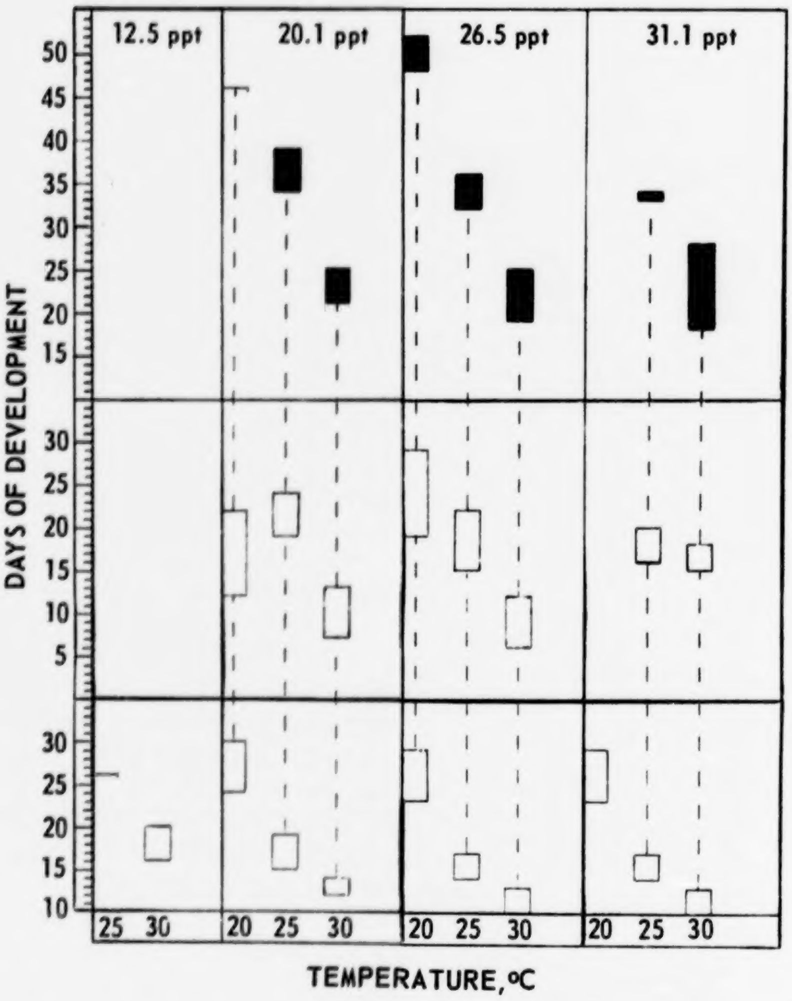

Figure 1. Comparison of minimum and maximum time of zoeal development (white), megalops development (stippled), and total development (black) to first crab stage for larvae of P. herbsti reared under different salinities and temperatures.

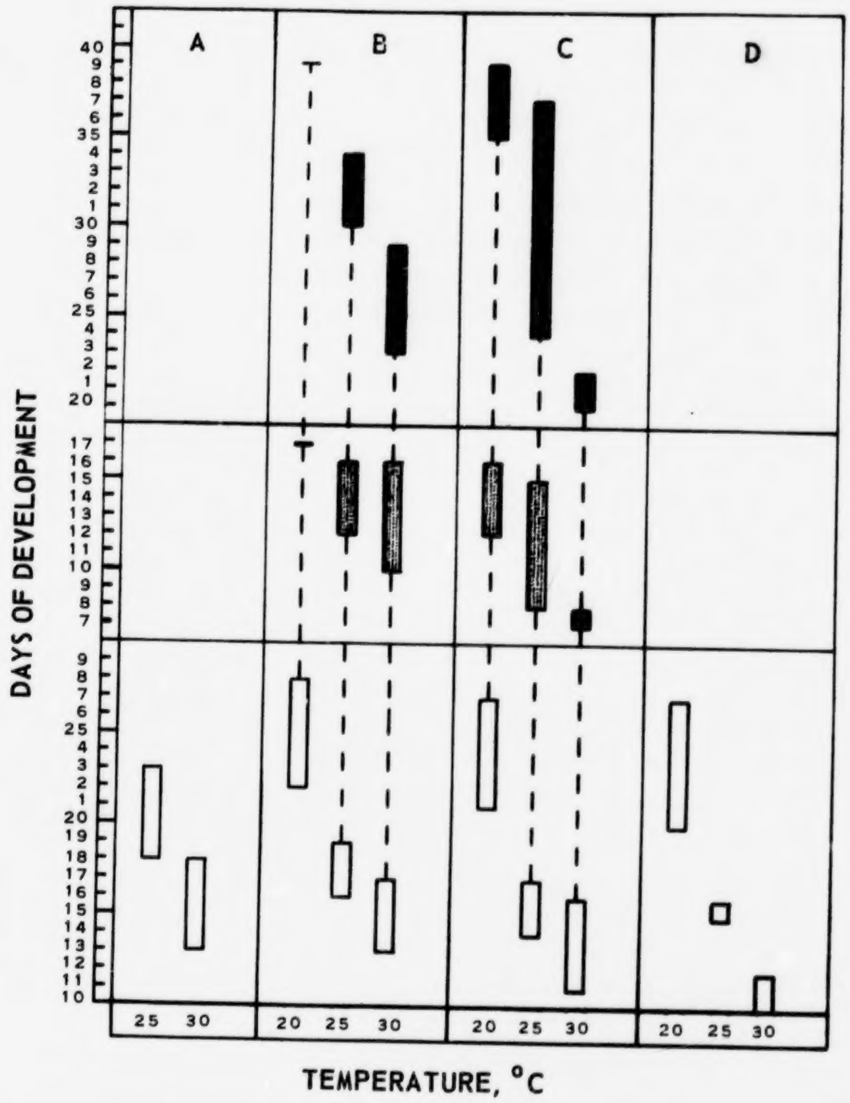

Figure 2. Comparison of minimum and maximum time of zoeal development (white), megalops development (stippled), and total development (black) to the first crab stage for larvae of S. cenereum reared under different salinities and temperatures.

is a gradual reduction in the time of development from low salinity to high salinity. As with the larvae of *P. herbsti*, the duration of the individual larval stages of *S. cinereum* at 30°C was approximately half the time required for comparable development when reared at 20°C.

While data are not as complete as with the other species studied, *Hepatus epheliticus* larvae reared under different conditions of salinity present a totally different picture. The adults of *H. epheliticus*, the "box crab," are normally found in shallow offshore waters of high salinity and are only occasionally collected in the inlets leading to the bays and estuaries. Although hatching of the larvae of *H. epheliticus* occurred at all five salinities shown in Table 1, complete development to the first crab stage was only observed at 30 and 35 ppt. At 30 and 40 ppt the larvae did not live to complete the first zoeal molt. Survival to the first crab stage was higher at 35 ppt (13%) than at 30 ppt (3%). The rate of development was also affected in the lower salinities: at 25 ppt the time for development to the megalops was 33 to 38 days, while at 30 and 35 ppt comparable development required 21 and 26 days, respectively.

The fifth species to be considered, *Callinectes sapidus*, presents still a different picture. Although the adult females are found in salinities as low as 0.1 ppt, the majority migrate to waters of higher salinity prior to ovulation and the ovigerous females are frequently taken in salinities of from approximately 22 to 35 ppt. Hatching of the larvae of *C. sapidus* was observed at all experimental salinities other than 10.5 and 15 ppt. In water of 20.1 to 32 ppt. The zoeas hatched as first stage larvae, and the so-called "pre-zoea" was never observed. Complete development to the first crab stage occurred in only three of the temperature-salinity combinations shown in Table 1: 25°C, 20.1, 26.7, and 31.1 ppt. There is some evidence that in water of 32 ppt a greater period of time is required for complete development to the first crab, but additional experiments are needed to confirm this. While zoeal development did not occur below 20.1 ppt more recent experiments have shown that the megalops can complete metamorphosis to the first crab when maintained in salinities as low as 5 ppt. Preliminary results suggest that in lower salinities a greater period of time is required for development of the megalops than in the higher salinities.

Table 1. SALINITIES AND TEMPERATURES UNDER WHICH LARVAE OF FIVE SPECIES OF CRABS WERE REARED IN THE LABORATORY.

	Temperature, °C	Salinity, °/oo	Number of combinations
R. harrisi	20, 25, 30	2.5, 5.0, 15.0, 25.0, 33.0, 35.0, 40.0	21
P. herbsti	20, 25, 30	12.5, 20.1, 26.7, 31.1	12
S. cinereum	20, 25, 30	12.5, 20.1, 26.7, 31.1	12
H. epheliticus	25	20, 25, 30, 35, 40	5
C. sapidus	20, 25, 30	10.5, 15.6, 20.1, 26.7, 31.1	15

Of the five species of crabs discussed, only one species showed any variation in number of larval stages. *C. sapidus* normally has seven zoeal stages and one megalops stage but in some experiments an eighth zoeal stage was found. This "extra" stage rarely completed development to the megalops and the few that did were never observed to metamorphose to the first crab stage. There is no evidence, however, that these "extra" stages are associated with either salinity or temperature.

SURVIVAL OF CRAB LARVAE

From laboratory experiments on the rearing of larvae, we now have relatively extensive data on the effects of salinity and temperature on the survival of the larval stages of three species: *R. harrisi*, *P. herbsti*, and *S. cinereum*.

Experiments with larvae of *R. harrisi* have shown that at a salinity of 1 ppt there was no survival beyond the second zoeal stage. At 2.5 ppt none of the zoeal stages reached the megalops at 20°C, but at 25°C and 30°C some did metamorphose to the first crab (Fig. 3). At 5 ppt there was a greater survival at 25°C and 30°C than at 20°C. At salinities of 15, 25, and 33 ppt there was a greater percent survival at 20° and 25°C than at 30°C (Figure 3). More recent experiments have shown that survival is even possible at 40 ppt, although the percentage is considerably reduced. One of the remarkable features about the development of these crabs is the relatively consistent high survival of all four zoeal stages to the megalops in the range of salinity from 2.5 to 33 ppt. There may be considerable mortality between the megalops stage and the first crab stage in some

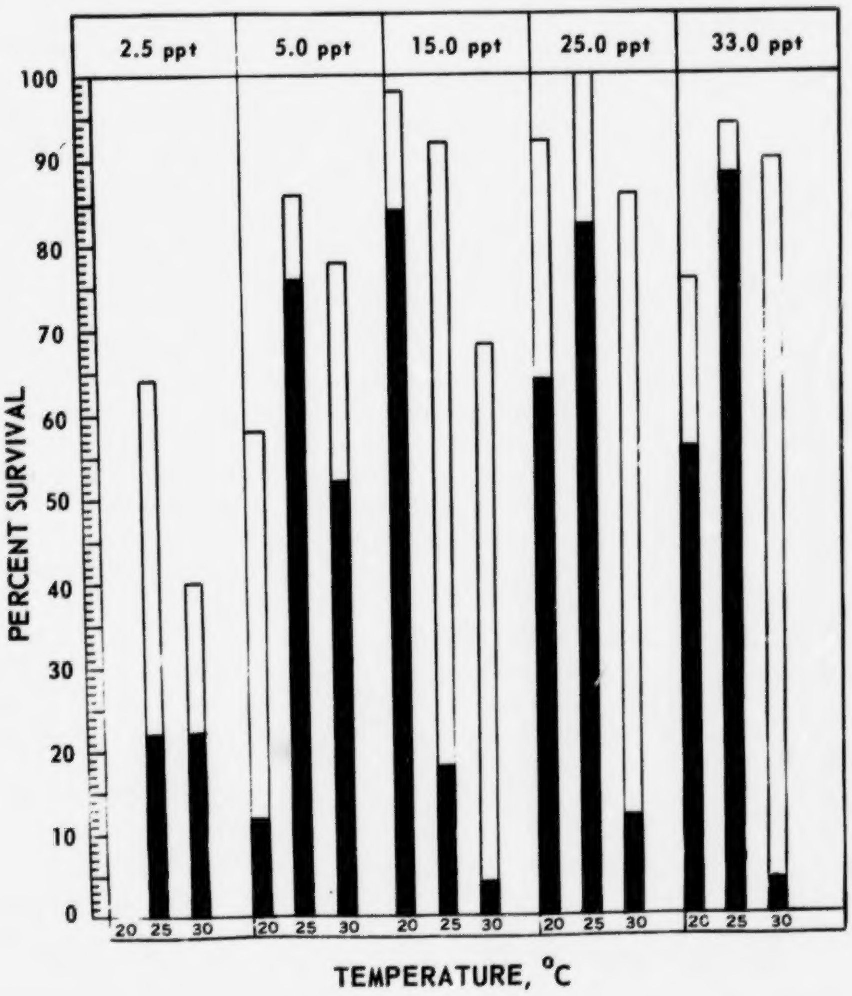

Figure 3. Percent survival to the megalops stage (white) and total survival to the first crab (black) for larvae of Rhithropanopeus harrisi reared at different salinities and temperatures.

salinity-temperature combinations but even so, a larger percentage of the eggs laid reach the first crab stage than those of any species that has previously been reared in the laboratory.

The larval stages of *Panopeus herbsti* displayed a similar survival (18%) in all salinities other than 12.5 ppt, 30°C (Figure 4). The highest percent survival was at the high temperatures and the lowest survival was observed at 20°C. There does not appear to be an "optimum" salinity-temperature combination for each zoeal stage nor was any one larval stage particularly sensitive to any one salinity.

Survival of the larval stages of *S. cinereum* was definitely affected by salinity. As shown in Figure 5 the first stage zoeae withstood the higher salinities (26.7 to 31.1 ppt) better than the lower salinities (12.5 to 20.1 ppt). Mortality in the second and third zoeal stages was relatively low at all salinities. In the fourth and last zoeal stage, however, 50 to 83 percent of the total mortality occurred at a salinity of 31.1 ppt, 30°C. In most cases the zoeae died in the process of molting to the megalops stage.

ESTIMATION OF OPTIMUM CONDITIONS

Although experiments on rearing of larval crabs under different conditions of salinity and temperature can contribute to our understanding of the environmental factors that affect development and survival, it is physically impossible to subject the larvae to all the combinations of salinity and temperature in which they would conceivable be found under natural conditions. It is possible, however, by statistical methods to postulate the response of larvae to a much greater variety of environmental conditions. With the considerable assistance of Dr. R. Monroe, Department of Experimental Statistics, North Carolina State College, Raleigh, N.C., we have attempted to estimate the distribution and mortality of larval stages of several species at the salinities and temperatures of the natural environment. The basic experimental design used is usually referred to as a factorial design. Specifically the plan was a 3 x 4 factorial in which three levels of temperature and 4 levels of salinity were used, making a total of 12 different combinations of the experimental conditions. If one assumes that the effects of temperature and salinity are continuous, and further that a temperature-salinity interaction may exist, then the twelve experimental combinations may be regarded as a sample in a continuum of temperature and salinity. One may also postulate the existence of a continuous response (i.e., percent mortality) as a function of temperature and salinity, and that either unique optimum combinations of the two factors may exist or that several combinations of temperature and salinity may produce the same response. The estimation of this functional relationship has come to be called "the fitting of a response surface" (Box and Youle, 1955).

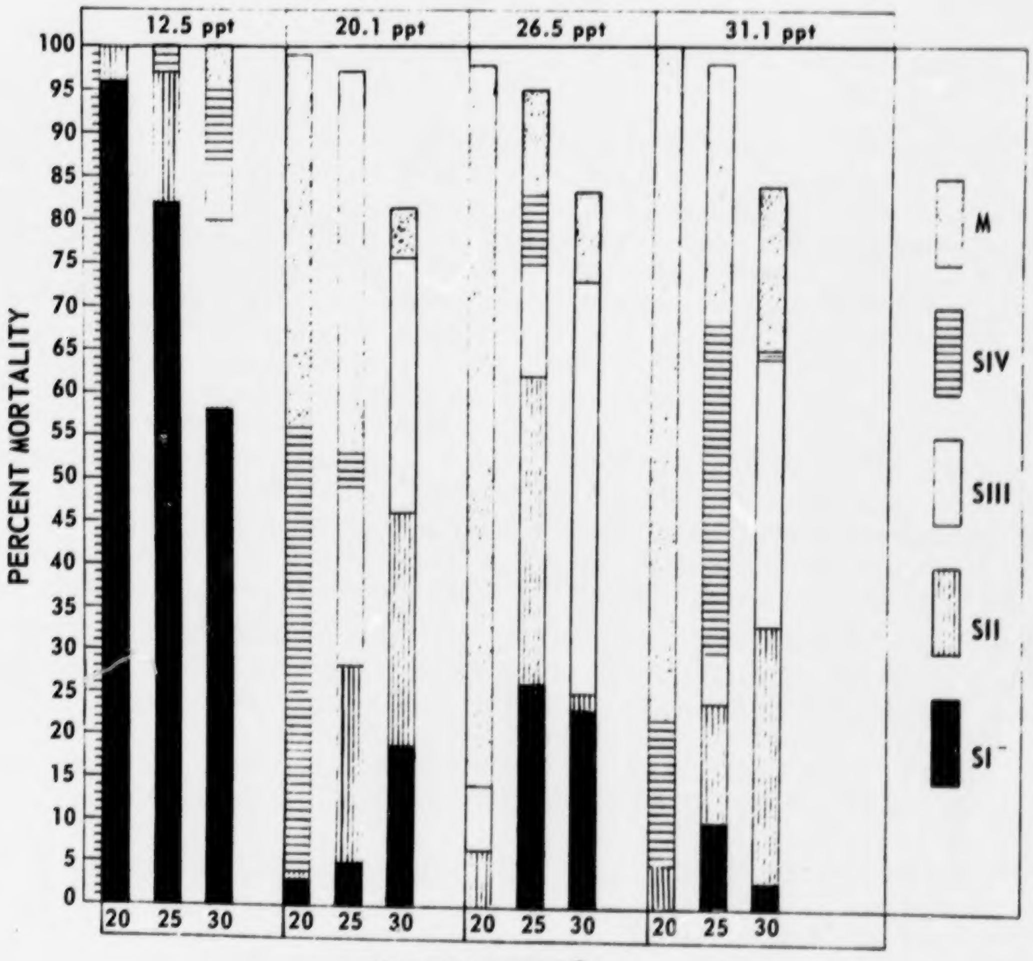

Figure 4. Percent mortality of each stage of P. herbsti reared at different temperatures and salinities. SI - SIV = zoeal stages I - IV M = megalops.

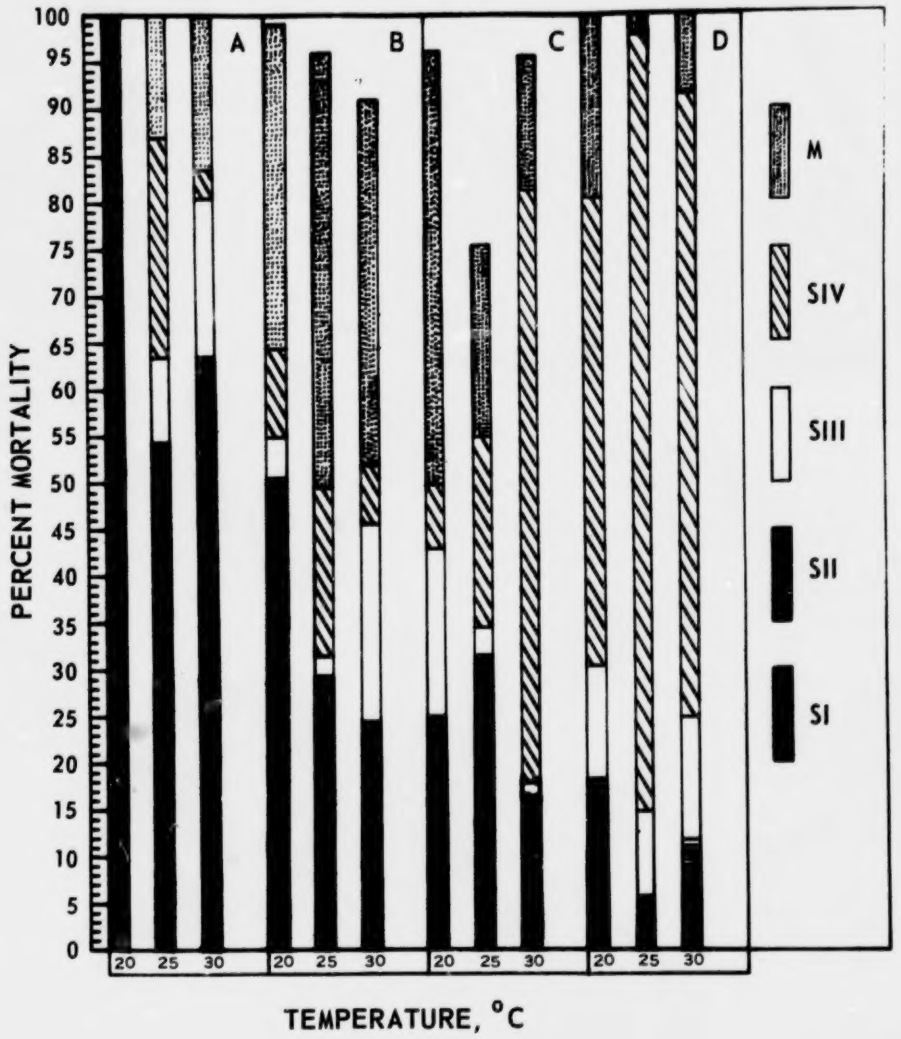

Figure 5. Percent mortality of each stage of S. cinereum reared at different temperatures and salinities. SI - SIV = zoeal stages I - IV: M = megalops.

We have attempted this treatment with data obtained from the rearing of *S. cinereum* and *P. herbsti* larvae, and are currently applying the same analysis to our data on the effect of salinity and temperature on development of *R. harrisi* larvae. More detailed information on the treatment, as well as the results, are available (Costlow, Bookhout, and Monroe, 1960 and 1962).

In general, the contours shown in Figures 6 to 10 represent the predicted values of temperature and salinity that would produce the mortality indicated by the contour lines. It should be emphasized that the usual dangers of extrapolation beyond the ranges of the observed data are as inherent in this method of prediction as in any other. The significance levels chosen in making individual tests of significance were 5 percent, 10 percent, and 20 percent as the exploratory nature of this study was to identify possible leads toward important factors or combinations of factors. Effects that were significant at the 5 percent level were labeled as "marked effects," while those at the 10 and 20 percent levels were denoted as "some effects." T and T2 were used to denote the linear and quadratic effects of temperature, S and S2 the same for salinity, and (T X S) the interation between salinity and temperature. A summary of these tests for the five larval stages of *Sesarma cinereum* is shown in table 2.

Table 2. SIGNIFICANCE OF SALINITY AND TEMPERATURE EFFECTS ON DEVELOPMENT OF THE FIVE LARVAL STAGES OF SESARMA CINEREUM REARED IN THE LABORATORY. T AND T^2, LINEAR AND QUADRATIC EFFECTS OF TEMPERATURE: S AND S^2, LINEAR AND QUADRATIC EFFECTS OF SALINITY: T x S, INTERACTION OF TEMPERATURE AND SALINITY. "MARKED EFFECTS" REPRESENTS SIGNIFICANCE AT THE 5% LEVEL AND "SOME EFFECTS" INDICATES SIGNIFICANCE AT THE 10% AND 20% LEVEL.

	Marked effects	Some Effects	Over-all Correlation
Stage I	S	T, T^2	0.872
Stage II		S, T^2	0.742
Stage III		T, T^2, S, (T X S)	0.829
Stage IV	S^2, (T X S)	S	0.877
Stage V	S	(T X S)	0.847

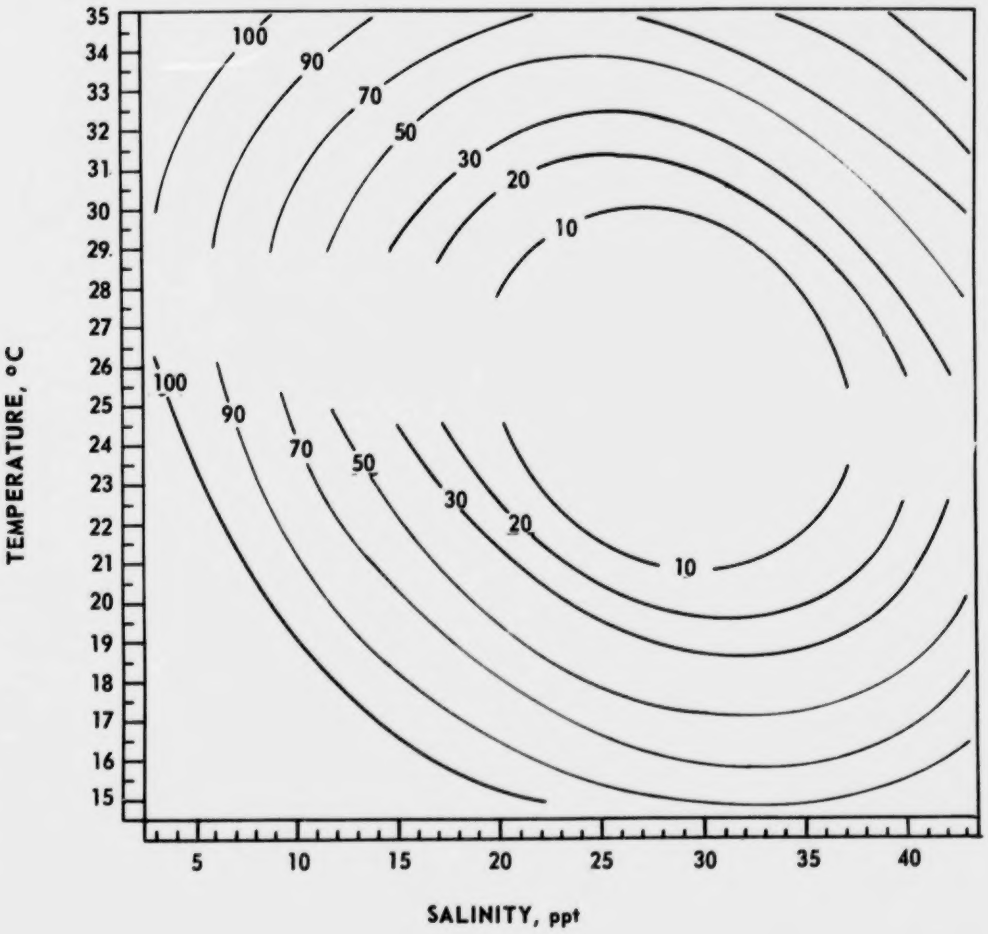

Figure 6. Estimation of percent mortality of first zoeal stage of S. cinereum based on the fitted response surface to observed mortality under twelve different combinations of salinity and temperature.

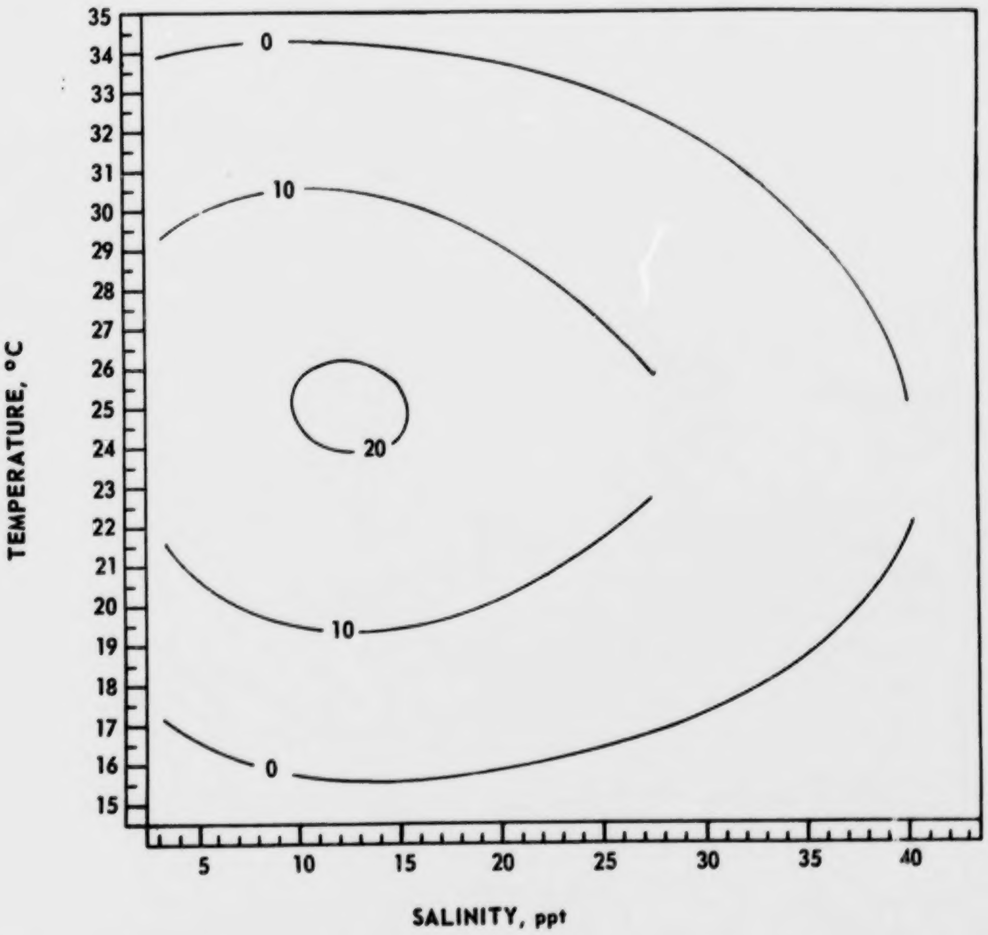

Figure 7. Estimation of percent mortality of second zoeal stage of S. cinereum based on the fitted response surface to observed mortality under twelve different combinations of salinity and temperature.

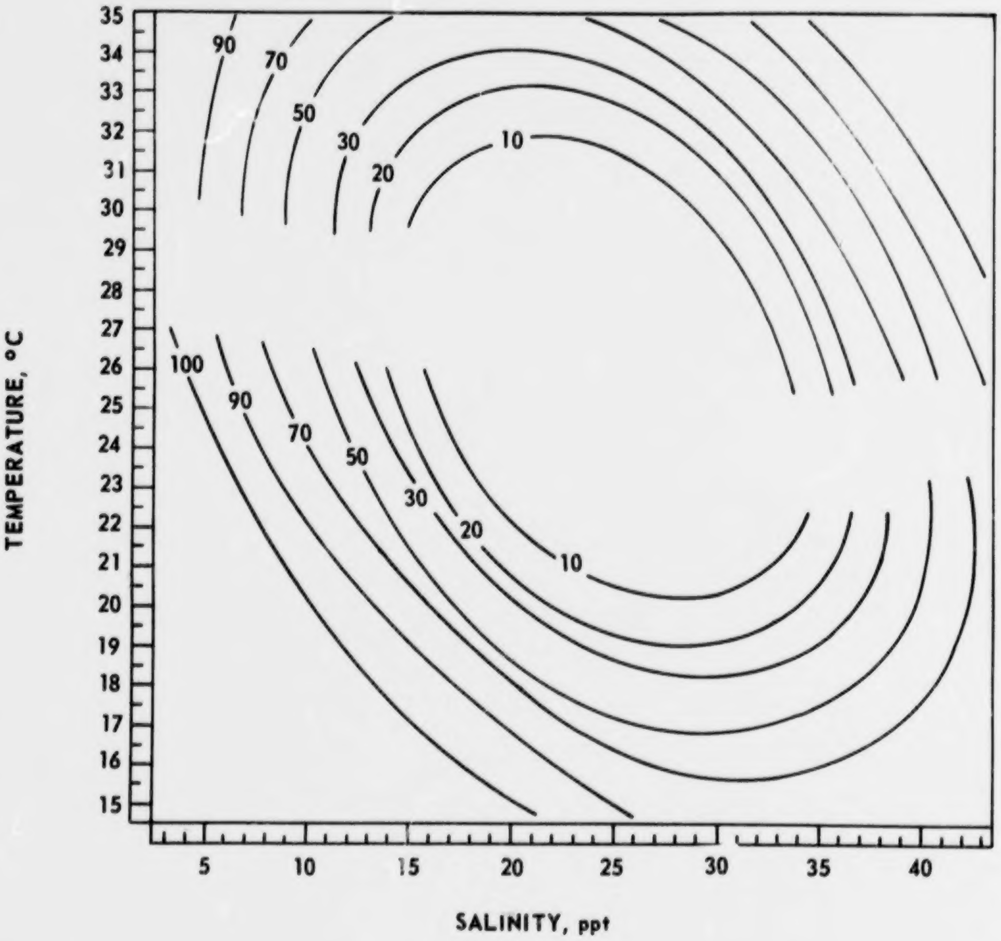

Figure 8. Estimation of percent mortality of third zoeal stage of S. cinereum based on the fitted response surface to observed mortality under twelve different combinations of salinity and temperature.

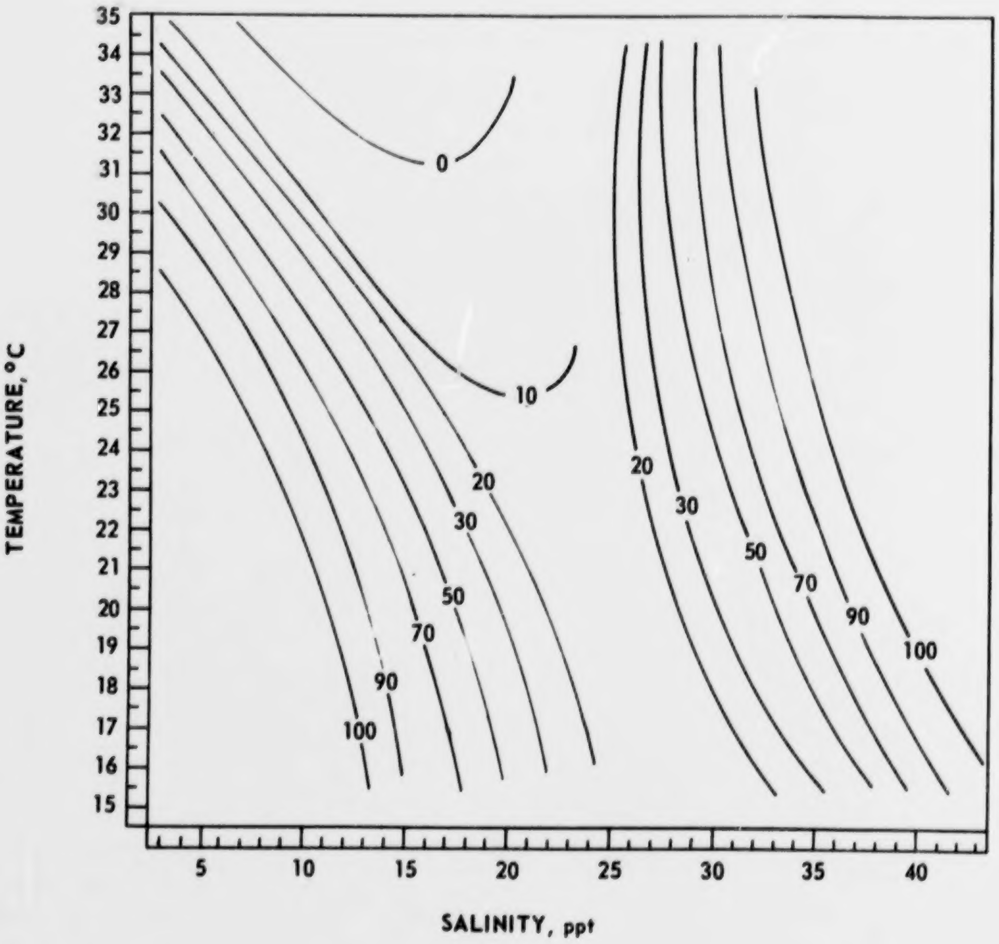

Figure 9. Estimation of percent mortality of fourth zoeal stage of S. cinereum based on the fitted response surface to observed mortality under twelve different combinations of salinity and temperature.

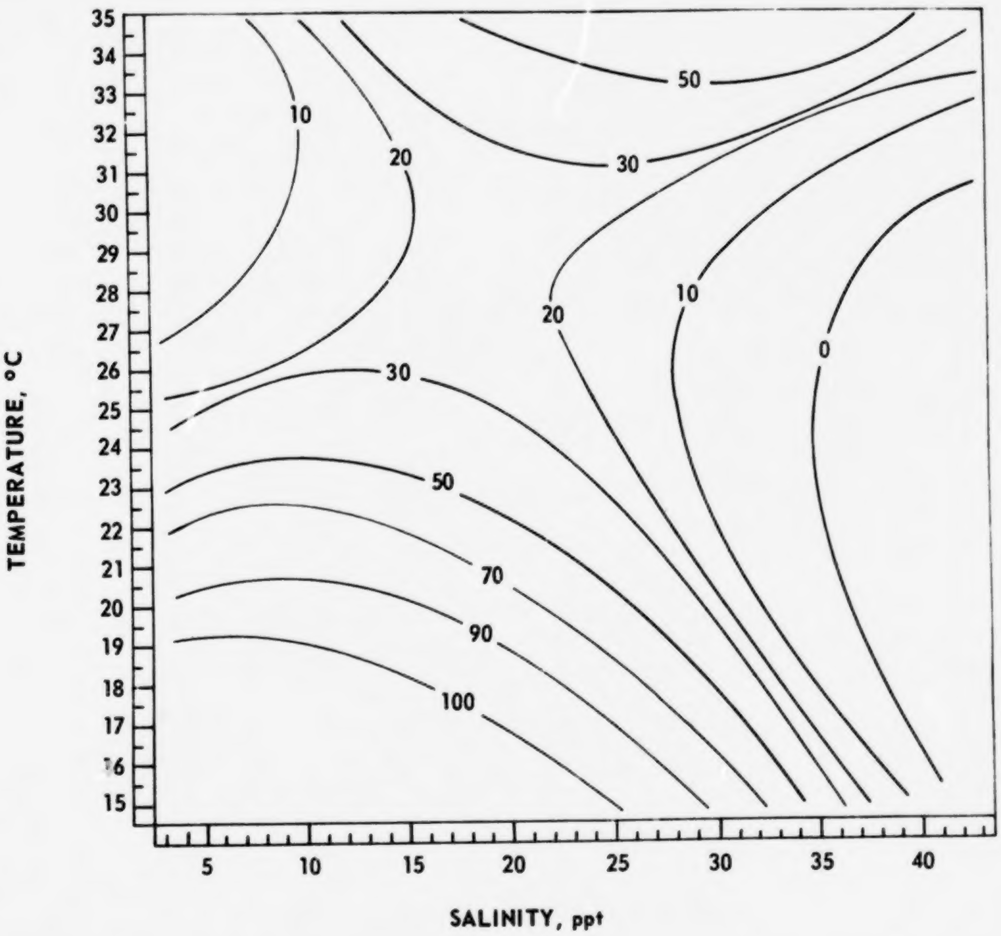

Figure 10. Estimation of percent mortality of megalops stage of S. cinereum based on the fitted response surface to observed mortality under twelve different combinations of salinity and temperature.

The results of this treatment give detailed information on predicted tolerance of the individual larval stages, and also provide some general information on larval distribution and survival. For example, the successful completion of the life history of *S. cinereum* appears to depend largely on the fourth zoeal stage. That is, survival and molting to the megalops can only occur in estuarine salinities. Those that are carried out to the higher salinities of the open sea, or trapped in tide pools of higher salinity, would not be expected to complete development. Once the megalops stage is reached, however, it can withstand the salinity of the ocean and a wide range of temperatures and it can also survive in lower salinities at higher temperatures. In contrast to larvae of *S. cinereum*, the larval stages of *P. herbsti* can develop successfully in a wider range of salinities through all stages of development.

One may also hypothesize that the effect of temperature on successive larval stages limits the productive spawning period of *P. herbsti*. The "spring brood" of larvae is favored by low water-temperatures; larval development is prolonged until warming conditions produce a favorable environment for the megalops stage. Larvae that hatched during the fall would not be so favored by the gradual reduction in water temperatures and mortality in the late zoeal stages and the megalops stage would be expected to be extremely high.

While numerous other comments would be possible on the ecological significance of these results and the use of this particular statistical treatment in studies of biological material, I will conclude with this one general comment. The rearing of marine animals, through all larval stages to the pre-adult, provides us with a tool for studies on morphology and taxonomy, physiology, endocrinology, parasitology, and ecology, which makes possible considerably greater insights into the basic environmental and physiological requirements of these animals. When we acquire a more complete understanding of the effects of environmental factors on rates of development, metabolic activity, and survival, we can seriously consider using some of these larvae as extremely sensitive bioassay animals for laboratory studies on water quality and the degree of toxicity in estuarine and marine environments.

ACKNOWLEDGMENTS

Figures 1 and 4 have been taken from "Salinity-temperature effects on the larval development of the crab, Panopeus herbsti Milne-Edwards, reared in the laboratory", Physiol. Zoology (Pub. Univ. Chicago Press), 35: 79-93, 1962. Castlow, J.D., Jr., C.G. Bookhout, and R. Monroe.

Figures 2, 5, 6, 7, 8, 9 and 10 have been taken from "The effect of salinity and temperature on larval development of Sesarma cinereum (Bosc) reared in the laboratory", Biol. Bull., 118: 183-303, 1960. Castlow, J.D., Jr., C.G. Bookhout and R. Monroe.

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ENVIRONMENTAL REQUIREMENTS OF SHRIMP

A. C. Broad *

The shrimp fishery of the South Atlantic and Gulf coasts, the most valuable one in the United States, began in its present form with the application of the otter trawl to shrimp fishing sometime between 1912 and 1915 *(2)*. This fishery is based entirely on catches of three species: *Penaeus setiferus*,(Linnaeus), *Penaeus aztecus* Ives, and *Penaeus duorarum* Burkenroad (see Burkenroad *(6)* for systematic treatment). Catches are made in the Carolinas, Georgia, Florida, Alabama, Mississippi, Louisiana, and Texas. Related penaeids support important fisheries in other parts of the world.

Apart from their obvious economic significance, penaeid shrimp are of interest to zoologists because, unlike the majority of higher crustacea (to which penaeids are related), they offer no brood-pouch protection to their eggs, which they shed freely in the water. The eggs usually hatch within a day or less *(14, 23, 28, 29, 34)*. The first free-living form is a nauplius, a stage typical of the life histories of phyllopods, copepods, barnacles, and other entamostracans, but usually passed in the egg in the development of malacostracans.

Research on commercial shrimp in the United States began with the reports of Spaulding *(37)* and Viosca *(40)*, *(41)* in Louisiana. In 1931 the U.S. Bureau of Fisheries started a research program that has been continued to the present. Work is also in progress in state laboratories and universities in all the shrimp-producing states. Reports have delt with life histories, larval development, systematics, distribution, and general ecology, but almost all have been fisheries oriented. Until recently, attention was devoted largely to *P. setiferus*, the common white shrimp, which originally was the only species of commercial importance *(42)*. As the brown shrimp, *P. aztecus*, and the pink shrimp, *P. duorarum*, have become more abundant in the total landings, however, some scientific attention has been devoted to them.

The life history of the white shrimp is fairly well known by now. The following summary is based on the work of many investigators *(1, 4, 5, 6, 7, 13, 17, 18, 29, 30, 31, 34, 42, 44, 46)* who are now in more or less substantial agreement on generalizations.

White shrimp are abundant in the United States from Cape Hatteras to Cape Canaveral and in the Northwestern and Western Gulf of Mexico. The adults spawn at sea, probably over the entire range of abundance, but usually in shallow, coastal water not in excess of 10 to 12 fathoms deep. Well-defined, seasonal spawning suggests a relationship with temperature on which most authors are in agreement, although specific effects of temperature on maturation or release of eggs are poorly known.

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The eggs, which are demersal in sea water, are probably shed while the parent shrimp is swimming at some intermediate depth, as has been observed in *Penaeus japonicus (28)*. They hatch within 12 hours. The newly hatched young, about 0.3 mm long, probably pass through five naupliar stages in 1 day *(34)*, but only three forms were found in Gulf of Mexico plankton by Heegaard *(22)*. The nauplii subsist on contained yolk. By the second day the young shrimp are in the first of three so-called protozoeal stages and feed for the first time. The protozoeal phase may last until the sixth day after hatching by which time the larvae are in the first of two (possibly three) so-called mysis stages. Metamorphosis to the postlarval stage occurs on the tenth to twelfth day, but the young postlarvae, now about 4.2 mm long, remain planktonic. Postlarvae of related penaeids *(23, 28)* molt every other day, and this may be reasonable estimate of the molting frequency of very young white shrimp. The larvae are found in oceanic plankton, presumably in the general area in which spawning occurs. Postlarvae of about 6 to 7 mm in total length, however, are abundant in and around inlets and estuary mouths. These individuals are in at least the second postlarval molt cycle. By means not understood but presumably by using tidal currents *(45)*, the young shrimp make their way to the less saline, shallower portions of estuaries where they adopt a benthonic existence, usually on muddy substrates.

Growth of juvenile shrimp is rapid. Estimates of this growth are based on three different procedures, each of which contains intrinsic bias: the advance of modes are limits in subsequent length-frequency distributions, observed growth of captive individuals, and growth of tagged individuals. On the basis of length-frequency data, Williams *(44)* estimates a growth rate of 36 mm per month for shrimp of about 70 mm total length. Lindner and Anderson *(31)*, based on the results (and extrapolations thereof) of tagging experiments, consider the length increment to be 80 mm in the first 2 months, 25 mm a month at an average size of 100 mm, and 15 mm a month at an average size of 125 mm. Growth estimates based on observations of postlarval shrimp in aquaria are on the order of 20 mm per month *(34)*. All growth estimates have been made for summer temperatures or are based on summer populations. Growth and other activities are retarded by cold.

According to the length-frequency plots of Lindner and Anderson *(31)*, Louisiana white shrimp about 140 mm in length are sexually mature, but it is a curious fact that minimum sizes of mature white shrimp have not been reported. Based on available growth data, white shrimp living in warm water would be sexually mature at an age of about 6 months. Gunter *(17)* based a similar conclusion on length-frequency data. The largest white shrimp may be 200 mm long, but growth, after about 18 months, is negligible. There now seems to be general agreement that white shrimp may spawn repeatedly in a single year and may live longer than 1 year.

Growth of juvenile shrimp is accompanied by movement back toward water of higher salinity. Correlations of size and salinity, at least within narrow ranges, have not been demonstrable, however. Maturing shrimp leave estuaries at all seasons of the year, but there is a general, and evidently temperature-related, exodus of all but the smallest shrimp in autumn. Juveniles may overwinter in dormancy in estuaries.

Lindner and Anderson *(31)* report a southward coastwise migration of Atlantic white shrimp in autumn and northward migration in the spring. There are corresponding seaward-shoreward movements in the Gulf.

Until recently, relatively little was known of the life history of the pink shrimp, *P. duorarum*. The following is based largely on the work of several investigators *(4, 6, 7, 10, 14, 15, 24, 25, 44, 46)*.

Pink shrimp are abundant in North Carolina (where they are not pink) and in the eastern Gulf of Mexico and occur in the remainder of the shrimp-producing region. The adults spawn at sea in water usually less than 28 fathoms deep and possibly only at temperatures in excess of 24°C *(15)*. The larvae resemble those of the white shrimp and, like them, are members of the ocean plankton. There are five naupliar stages, three protozoeal forms, and three mysis forms. The eggs hatch within a day, and the nauplier phase lasts only 24 hours *(14)*. The frequency of subsequent molts is unknown. The poltlarvae are about 4 to 5 mm long at the time of metamorphosis and still members of the ocean plankton. They subsequently enter estuaries and have been found on nursery grounds in North Carolina at lengths of 17 mm or less *(43, 44)*. The nursery areas of pink shrimp may be more saline and have coarser sedments than those of white shrimp.

Growth is rapid. Various estimates from 20 to 52 mm per month have been based on length-frequency distributions. Eldred et al *(15)* estimates an average lenght of 75 mm after 4 months, 133 mm after 10 months, and 142 mm after 1 year. Williams *(44)* suggests a growth increment of 52 mm per month for juveniles. Cummings *(10)* found sexually mature pink shrimp 93 mm long. According to the available growth data, these may have been on the order of 6 months old. The largest individuals, however, are about 200 mm long and of unknown age.

The shrimp move seaward as sexual maturity approaches. The largest adults are always found some distance from shore. Unlike *P. setiferus*, which is diurnal, *P. duorarium* is nocturnal in habit and usually burrows into the bottom during the daylight hours.

Almost nothing is known of the life history of *P. aztecus*, the brown shrimp, but there is little reason to suspect that the generalities of its life history differ markedly from those of other penaeids. Brown shrimp are abundant from North Carolina to Florida on the Atlantic Coast and in the western Gulf of Mexico. Williams *(44, 45)* and Bearden *(3)* found postlarvae abudant in the Carolinas from January or February through May or June and present all during the summer. Gunter *(17)* found small juveniles during most of the year in Texas, and Pearson *(34)* reported planktonic postlarvae, presumably of this species, year around in Florida and Louisiana. The young prefer nursery areas more saline than those of white shrimp *(18)*. The brown shrimp also prefer nursery areas with mud bottoms *(46)*. Growth of juveniles has been estimated at about 45 mm per month by Williams *(44)*. Adults 200 mm long are not uncommon. Burkenroad *(6)* states that, after a littoral youth, brown shrimp retire permanently to deeper water offshore. Spawning, at least in the Gulf, may occur almost continuously. *P. aztecus* shows some tendency toward the nocturnal habit of *P. duorarum (29)*.

In spite of the general similarities in the life histories of commercial shrimp, there are probably specific differences. These differences are still difficult to define, except generally. Springer and Bullis *(38, 39)* and Hildebrand *(24, 25)* found differences in the distribution of adults in the Gulf: They found white and brown shrimp on terrigenous silt bottoms, and pink shrimp on coral sand and shell. White shrimp occur mainly inshore of the 10 fathom curve, and brown shrimp offshore of it. White and brown shrimp are found largely in the western Gulf, and pinks are appropriate bottoms in the eastern Gulf. The invertebrate and fish associates of the pink and brown shrimp differ *(24, 25)*. Similar separation of adult populations occurs in the Atlantic *(4)*.

The distribution of shrimp in the Gulf may be associated with differences in the nursery areas adjacent to the eastern and western portions. Hildebrand *(25)* suggests that a combination of low salinity and mud bottom may bar *P. duorarum* from Louisiana west of the Mississippi, but Springer and Bullis *(38)* and Darnell and Williams *(12)* found a few pink shrimp in Louisiana. Gunter *(18)* suggests that salinity of Texas nursery areas may be optimal for *P. aztecus* and that of Louisiana estuaries for *P. setiferus*. Williams *(46)* demonstrated that, if offered a choice, young pink shrimp will select coarse substrates and young whites and browns will settle on mud.

In spite of the obvious significance of salinity to shrimp life histories, the relationship becomes extremely confusing when one attempts to do more than generalize. All investigators agree that smaller individuals occur in lower salinities, but attempts to demonstrate a relationship have met with little success *(31)*. Gunter *(17)* treats salinity in broad ranges: less than 20 o/oo; 20 to 29.9 o/oo; and 30+o/oo, and shows a graphic relationship between size of white shrimp and these three salinity ranges. The smallest shrimp, however, occur at all salinities and Gunter himself *(18)* cites a collection of white shrimp 15 to 66 mm long from the Laguna Madre in Texas taken at a salinity of 41.3 o/oo. Williams *(45)* reports the salinity of nursery areas in North Carolina as "nearly fresh to 37 o/oo." Johnson and Fielding *(29)* reared *P. setiferus* postlarvae at 31.2 to 37.1 o/oo and, in one experiment, obtained better growth at 34 o/oo than at 18.5 o/oo. Lunz *(32)* reared juvenile white shrimp at 17.6 to 33.2 o/oo.

Data on salinity relations of pink and brown shrimp, though fewer, are equally inconclusive. Darnell and Williams *(12)* took juvenile *P. duorarum* from Lake Pontchartrain, Louisiana, where the salinity range was of 3.9 to 10.3 o/oo. Williams *(44)* although he did not mention salinities of specific collections, found juveniles of all species within his range of nearly fresh to 37 o/oo, occupying the same nursery areas, but mainly at different times of the year. Gunter *(17)* took 4,201 brown shrimp from water of less than 10 o/oo, 7,510 from water of 10 to 20 o/oo, 2,920 from water 20 to 30 o/oo, and 1.010 from water of 30+ o/oo salinity. These data illustrate the hopelessness of relating the available collection data to the effects of salinity on shrimp. More acceptable is the estimate of Gunter *(18)* that optimum salinity for juvenile *P. setiferus* is less than 10 o/oo (and, presumably, that the optimum salinity for *P. aztecus* nursery areas is in excess of 10 o/oo). Hildebrand *(25)* suggests an optimal salinity of approximately 20 o/oo for *P. duorarum* nursery areas. By far the most satisfactory demonstration of a relationship between salinity and species of shrimp is a correlation between the catch of white shrimp in Texas with the annual rainfall for the previous 2-year period *(19, 26)*.

The work of Hudinage *(28)* on larval *P. japonicus* and that of Rao *(35)* on an Indiana penaeid, *Metapenaeus monoceros*, shed some light on salinity relations. Absolute values are not important to this discussion, but a definite range of salinity within which postlarval *P. japonicus* were able to survive was demonstrated by Hudinaga. Rao determined oxygen consumption of *M. monoceros* of various sizes when subjected to sudden changes in salinity. His material was obtained from water of either 33.5 o/oo or from 20 o/oo salinity and subjected to salinities of 33.5 o/oo, 16.75 o/oo, and 8.4 o/oo, and to tap water. In each case of osmotic stress, oxygen consumption, as might be expected, increased, but the increase was inversely proportional to the size of the shrimp. Thus, juveniles placed in water of salinity other than that in which they were collected were in a greater stress situation than were adults similarly treated. Although salinities other than those within an optimal range may not kill shrimp, the osmotic stress so imposed may result in increased energy demands on juveniles.

Some temperature effects on shrimp are well known, and some other data have been presented. Cold will kill shrimp *(19, 31, 33)*, but rapidity of temperature change seems to be more important than the final temperature. *(19)* Eldred et al. *(15)* report that *P. duorarum* were killed by 12.8°C in tanks and narcotized at 13.3°C, but Williams *(45)* found the same species living at 4°C, but inactive below about 15°C. Lindner and Anderson *(31)* suggest that growth of white shrimp does not occur below 20°C. Spawning of all species is probably associated with temperature or with trends in temperature, as are movements and migrations

(31), but specific effects are unknown. There are no data on effects of high temperatures on U.S. penaeids. Scholander et al.*(36)* found that *P. brasiliensis* (presumably collected in Panama) did not survive at 35°C in closed respirometers, but Williams *(45)* and Lunz *(32)* found shrimp living at 35° and 36°C. Scholander's value of 35°C may have little meaning outside of the respirometer flask, but is significant that the range of temperatures within which his shrimp survived (15° to 30°C) was narrower than that of other tropical crustaceans tested. Q10 values, defined as the rate of change (slope) at giving temperatures of the curve of oxygen consumption plotted against temperature, were uniform (2.4) from 15° to 25°C, but decreased (1.7) at 30°C. Tropical *P. brasiliensis*, thus, is active within a relatively narrow temperature range. The similarity of the lower activity limit of *P. brasiliensis* to those observed by Williams and Eldred et al. on cold narcosis of *P. duorarum* may indicate similar thermal limits in U.S. penaeids. The reported observations of temperatures at which shrimp are collected seem to support this.

Shrimp are considered omnivorous by most authors. Weymouth et al.*(42)* observed cannibalism in captive shrimp. Foods reported either after observation or as a result of stomach analysis are listed with references:

Blue-green algae	*(15, 16)*
Green algae	*(15, 34, 44)*
Diatoms	*(28)*
Red algae	*(15)*
Leaves and other parts of vascular plants	*(11, 15, 16, 44)*
Dinoflagellates	*(15)*
Faraminiferans	*(11, 44)*
Sponge	*(16)*
Coral	*(16)*
Bryozoans	*(16)*
Nematodes	*(15)*
Polychaetes: jaws, setae	*(15, 44)*
Angleworms	*(34)*
Crustaceans, mandibles, chitin fragments, water fleas, copepods, ostracods, mysids, isopods, amphipods, penaeid shrimp, shrimp eggs, caridean shrimp, shrimp meal, *Artemia* nauplii	*(11, 15, 23, 28, 44, 46)*
Insect remains	*(11)*
Molluscs: shells, squid suckers	*(11, 15, 44)*
Fish: whole fish, fish meal, fish eggs, ribs, eyes, scales	*(15, 29, 34, 44)*

All authors who have examined shrimp stomachs eport organic debris and sand. Williams *(44)* found tomachs of juveniles empty in winter but always alf full to full in the autumn.

Although there is tacit agreement that the oxygen equirements of white shrimp are higher than are ose of browns or pinks *(46)*, no studies of oxygen consumption by U.S. penaeids have been attempted. Rao *(35)* has shown higher consumption per gram in young *Metapenaeus* in situations of osmotic imbalance. Scholander et al. *(36)* obtained oxygen consumption values for *P. brasiliensis* not significantly different from those of other tropical crustaceans tested, but noted a marked decrease in rate of oxygen consumption in closed respirometers at 30°C.

Butler *(8)* and Chin and Allen *(9)* have reported on the effects of insecticides on commercial shrimp. The data presently at hand indicate toxic effects of Endrin, Sevin, DDT, Dieldrin, Toxaphene, TDE, and Heptachlor in order of decreasing toxicity. Butler *(8)* reports tolerance limits of medium-sized *P. aztecus* ranging from only 2.5 ppb Edrin to 700 ppb Heptachlor. Smaller shrimp and postlarvae evidently are more sensitive. White shrimp generally show less resitance than browns.

By way of summary it might be pointed out that although we now seem to know a great deal about the life histories of commercial shrimp the information at hand is not sufficiently specific to talk, other than in general terms, about environmental requirements. All authors agree that there are two critical periods in the life history: the so-called first protozoeal stage when feeding commences and the postlarval period or early juvenile phase when the young shrimp are found in brackish, estuarine nursery areas. Available studies indicate possible specific differences in environmental requirements during the juvenile phase. *P. setiferus*, which has been shown to be most sensitive to some insecticides, evidently prefers (or is able to survive better in) low-salinity nursery areas. It is likely that oxygen utilization is also highest at this time, when the shrimp are in waters most likely to be polluted by industrial or domestic wastes, by run off from the land, or by insecticides from control programs. *P. aztecus*, which is evidently somewhat better able to withstand at least some kinds of pollution and probably has an overall lower oxygen requirement than the white shrimp, is also successful in somewhat higher salinities or rears successfully farther from terrestrial run-off than the white shrimp. *P. duorarum* may represent a still further step in this progression, but this has not been demonstrated. On the basis of available information, it does not seem unreasonable to assume that *P. setiferus* and *P. aztecus* are competitors during all or a good part of their lives. Such statements have been made by Williams *(45)* for nursery areas and are implicit in the writings of Gunter and Hildebrand *(19, 26)*. *P. setiferus* cannot compete with *P. aztecus* in high-salinity nursery areas 18 and may be less able to survive reduced oxygen tension or pollution as well.

Weymouth et al.*(42)* and Anderson et al.*(1)* estimated that the overall catch of the shrimp fishery of the South Atlantic and Gulf was 95 percent *P. setiferus* in the years perior to 1949. As long ago as 1950 a change in the shrimp catch in North Carolina was noted *(4)*. By associating species caught with time of the year, we were able to show that the catch had changed from predominantly white shrimp during the 5-year period 1940 through 1944 to predominantly brown shrimp in the period from 1945 to 1949 *(17, 21)*.

Until about 1948 the bulk of the shrimp catch in the Gulf of Mexico was *P. setiferus*, but in the years between 1948 and 1953 there was a decline in the abundance of white shrimp *(17, 21)*. Hildebrand *(24)* comments on the decline of the white shrimp landings in Lousiana and Texas and the increased landings of brown shrimp in Texas after 1948. Gunter *(18)* similarly compares white and brown shrimp landings in the western Gulf, which he relates to salinity differences between estuarine nursery areas in Louisiana and Texas. The increased catches of brown and pink shrimp may be largely a reflection of the opening of new grounds as suggested by Hedgepeth *(21)* but the overall decrease in the population of white shrimp is real. It seems reasonable to consider the possibility that slight changes in nursery areas may have occurred since the mid-1940's. The available data suggest that increases in salinity (possibly associated with decreased rainfall), decreases in oxygen (possibly resulting from increased demands on the environment by domestic or industrial organic wastes), or increases in the concentration of pesticides (associated with increased agricultural use of insecticides) would be disadvantageous to *P. setiferus* or might give the relatively hardier *P. aztecus* an advantage over the white shrimp. The magnitude of change that could be effective, although presently impossible to estimate, presumably could be very small. In view of the importance of the resource it seems that the area of competition between the species is a critical one as is the obvious need for better understanding of the basic physiological effects of the environment, especially on the juvenile shrimp.

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REACTION OF ESTUARINE MOLLUSKS TO SOME ENVIRONMENTAL FACTORS

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INTRODUCTION

For nearly 25 years, this laboratory has been concerned with identifying the environmental factors that permit our commercial species of mollusks to flourish. Studies have been conducted of Santa Rosa Sound, Florida, in adjacent areas known to be suitable for oysters but which support a relatively small commercial harvest because of the high concentration of natural predators. Santa Rosa Sound is approximately 40 miles long and presents a typical estuarine habitat, although it has two openings into the Gulf of Mexico. Land drainage into the Sound is sufficient to cause marked seasonal and tidal fluctuations in salinity.

In the past, too many biological studies on oysters have been in the nature of crash programs, limited in time and, more often than not, concerned with elucidating problems that had ceased to exist. The convenience of this location and the presence of a permanent staff made it possible to initiate a long-term program to observe the normal range of environmental factors in conjunction with the fluctuations in a natural population of oysters.

The unique facility of estuarine animals to adjust physiologically to more or less drastic environmental changes does not imply that they are equally successful under all conditions, but only that sufficient numbers survive to perpetuate the species. This was well demonstrated about 15 years ago when the upper Chesapeake Bay experienced a protracted period of flooding that extended into the mid-summer months. The oysters become, quite literally, bags of water and contracted perhaps a third in body size. Still, a very few managed to elaborate gametes, there was a late summer spawning, and the brood survived (Butler, 1949).

Our studies in Florida have undertaken the collection of 'routine' data to determine what are normal conditions, and to delineate if possible, changes in the oyster population associated with specific changes in the physical or chemical environment. This report is a brief summary of some of our observations in the past decade, what the environment has offered, and how the oyster and other estuarine animals have responded.

ENVIRONMENTAL CONDITIONS

There are certain classical parameters used to characterize the estuarine environment and we did not neglect these while searching for more obscure factors. Continuous recordings of fluctuations in water temperature have been maintained since 1947. Water samples have been collected at 4-hour intervals and titrated for total salts with Collier's water sampler (1953) for as long a period. Graphical summaries of these data show the average picture for the years 1951 through 1960 (Figure 1). Figure 2 shows the data for 1959, the year of lowest average salinity, and Figure 3 shows 1954, the year of both highest temperature and highest salinity in the 10-year period. Other specific factors in the environment were scanned from time to time, and these are considered in different sections of this report.

GROWTH

The most easily measured response of the animal to its surroundings is its growth, and the rate at which it grows may be of fundamental importance in evaluating the environment. One of our earlier studies (Butler, 1952) reported the seasonal percentage increase in length of a small population of oysters. These oysters, collected when less than a millimeter in length at metamorphosis, were observed as individuals for more than 3 years. Table 1 shows their seasonal increments and the change in the pattern of growth as they aged. When juveniles the greatest growth was in the summer. In their second year, there was an emphasis on winter growth, and in their third year almost no growth took place in the warm summer months.

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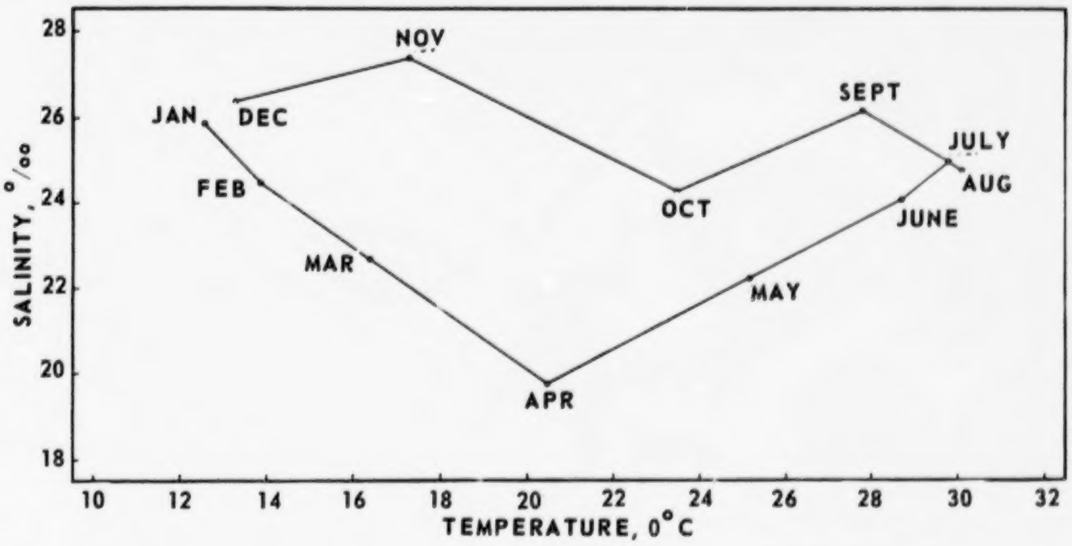

Figure 1. Comparison of the monthly water temperature and salinity in Santa Rosa Sound. Data are averages of continuous temperature recordings and titrated salinity samples collected at 4-hour intervals from 1951 through 1960.

Table 1. SEASONAL PERCENTAGE INCREASE IN LENGTH OF A SMALL POPULATION OF OYSTERS

Age of oysters, years	Percent of annual increment		
	June-September	October-January	February-May
1	46	36	18
2	14	52	34
3	11	68	21

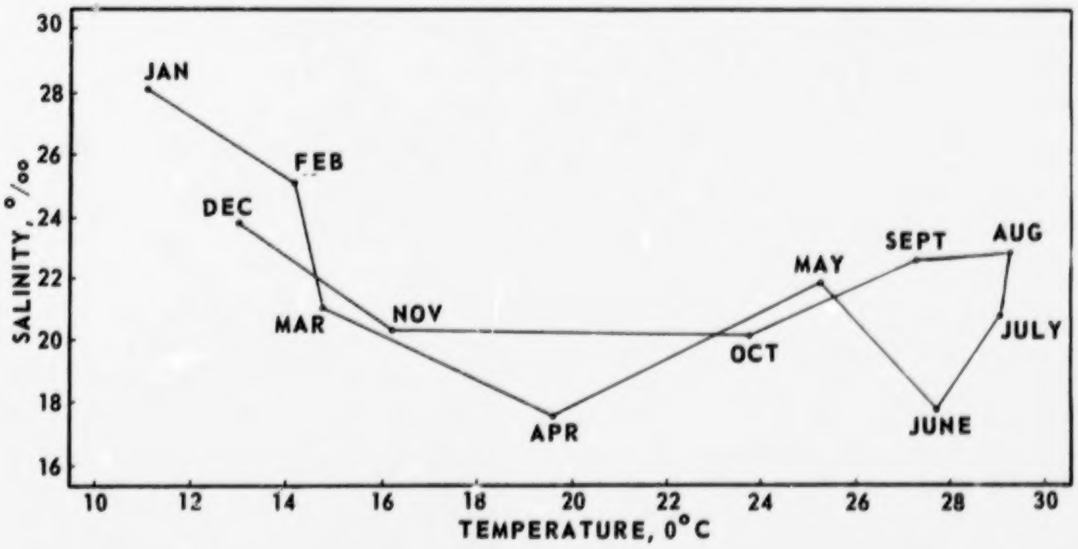

Figure 2. Comparison of the monthly water temperature and salinity in Santa Rosa Sound in 1959, the year of lowest average salinity in the 1951 through 1960 period.

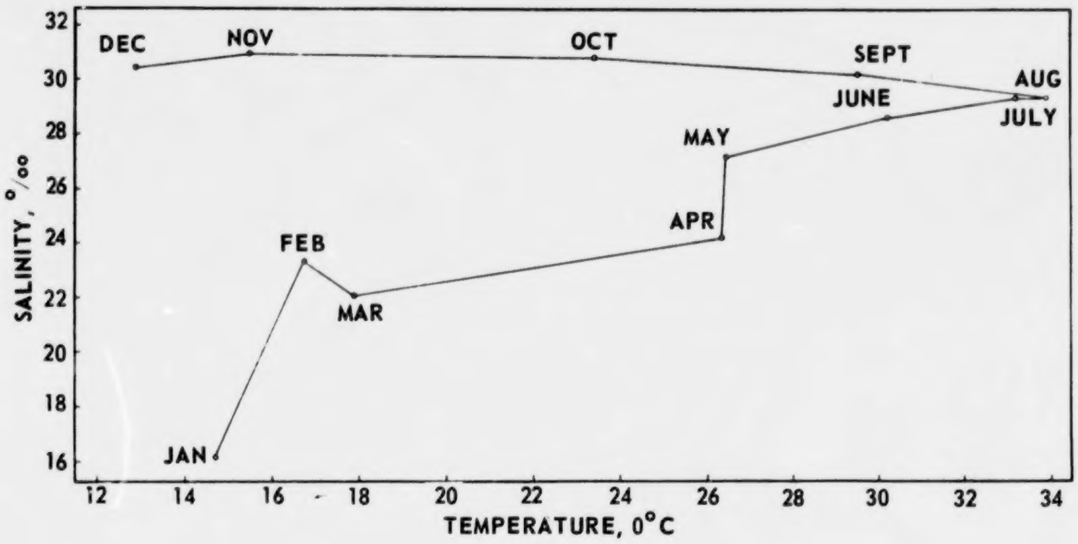

Figure 3. Comparison of the monthly water temperature and salinity in Santa Rosa Sound in 1954, the year of highest average salinity and highest water temperature in the 1951 through 1960 period.

There are several possible explanations for this changed pattern. It is typical of course for metabolic activity to decline with older animals, and it might be suggested that this combined with the demands of summer spawning is sufficient reaseon. In New England waters, however, growth and spawning are not antagonistic in timing, nor is it likely a process of aestivation, as I once assumed, at our summer temperature of about 90°F, since a similar decrease in mid-summer growth is reported by Medcof (1961) in the Canadian oyster at summer temperatures in the neighborhood of 75°F. The important point here is that with the rapid growth of shell in southern oysters it is frequently difficult to age an animal. Growth observations involving experimental animals of the same size but of different year classes may produce paradoxical results.

There are other features of the growth response in mollusks that make this at times an ambiguous criterion of the environment, to say the least, unless considerable background data are available. I should like to cite some examples that bear on this point.

The effect of temperature on growth rates may be seen in some Rhode Island hard clams *(M. mercenaria)* sent to our laboratory in the fall. The effect is dramatically shown in these clams by the white shell (Figure 4), which represents their growth when placed in a clean sand bottom after having been grown in a muddy area. Approximately 75 percent of this new shell was formed during the months of December and January, when had the clams remained in their native habitat they would have been hibernating. This is the simplest and most obvious growth-rate response on the part of the mollusk to one environmental factor, temperature.

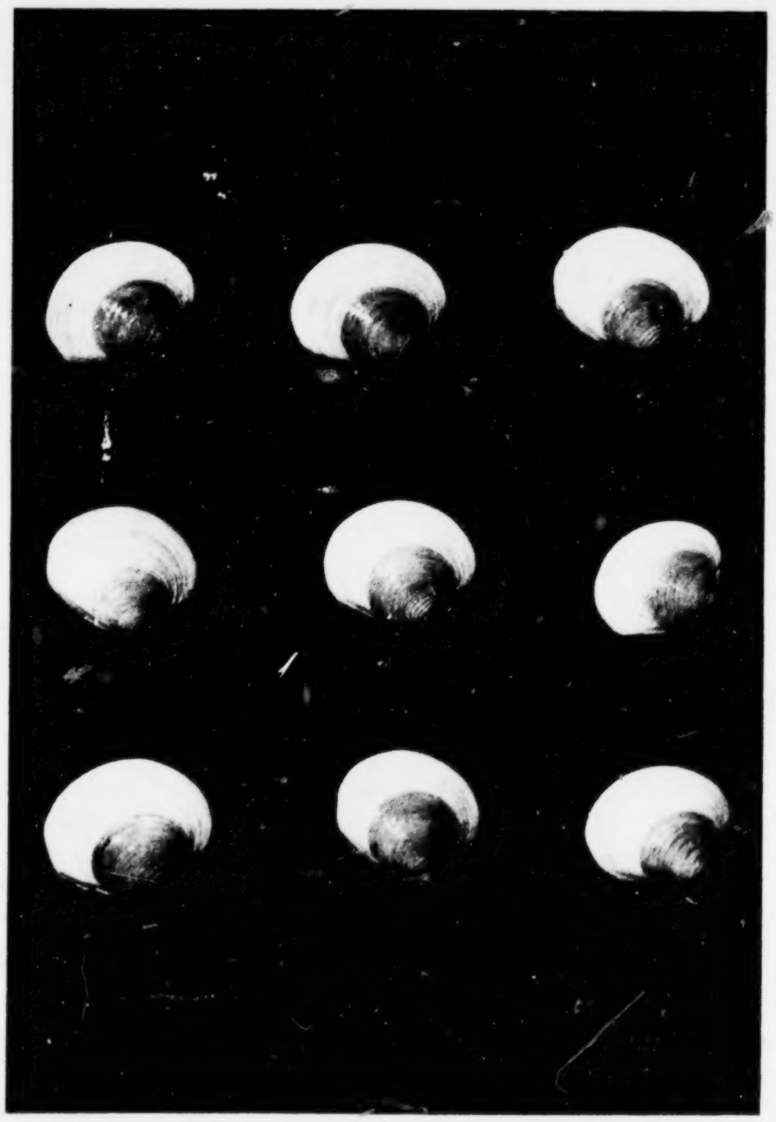

Figure 4. Hard clams, Mercenaria (Venus) mercenaria, transplated from Rhode Island to Florida in the fall were approximately one-half inch in diameter. They doubled in size in the winter months in Santa Rosa Sound when they would normally have been in hibernation.

A less obvious response is shown in the hard clams depicted in Figure 5. These clams, produced in the Bureau of Commercial Fisheries Biological Laboratory at Milford, Connecticut, by artificial propagation, were sent to Florida when approximately one-quarter inch in diameter. They were reasonably

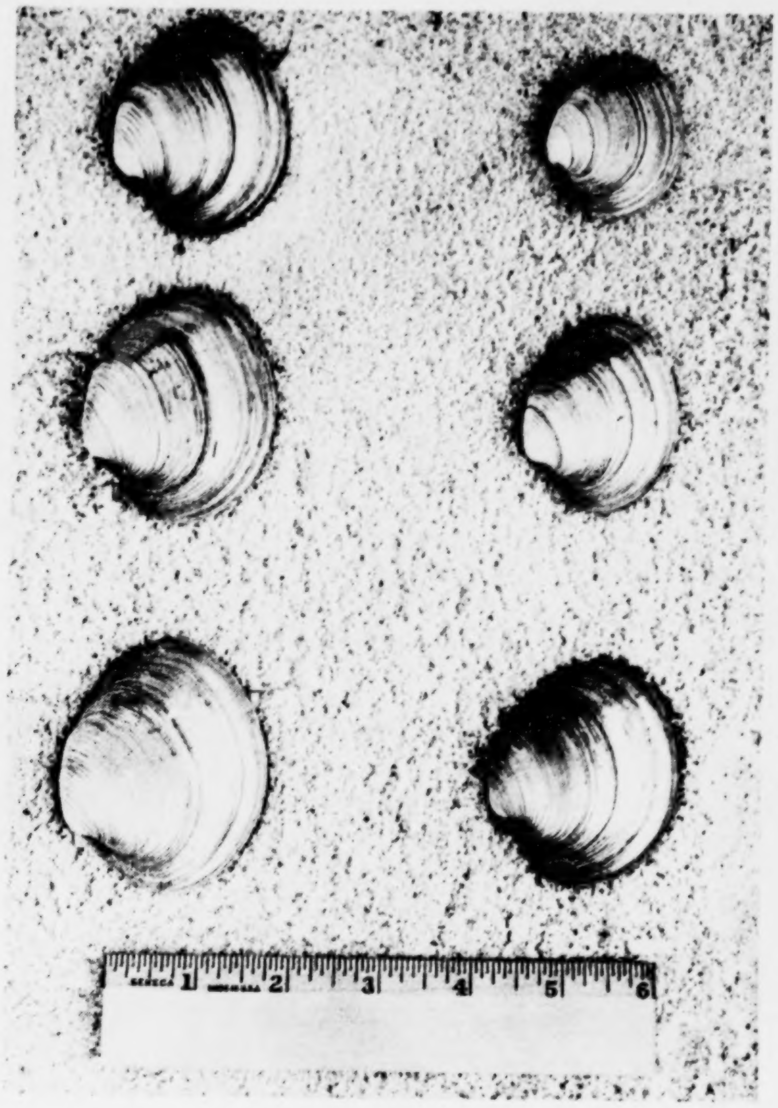

Figure 5. Hard clams, M. mercenaria, derived from a laboratory culture and of uniform size when planted. Individuals, from top to bottom, represent smallest, median, and largest clams recovered from the two locations at end of second year.

uniform in size, but were sorted into two lots whose average lengths were nearly identical. These two lots were planted in protected boxes on either side of the laboratory island and maintained for 2 years. From the biologist's point of view, the two stations were as nearly similar as one could select in the natural environment.

At the end of the 2 years, although mortality had been negligible in the two groups, there were very significant differences in the size range and average size in the two groups. The larger-size group (east) produced 24 percent greater volume of meats when the clams were harvested.

The boxes containing these clams were suspended from the docks at midwater and covered with hardware cloth. As such, they offered a protected niche for a variety of other sand-dwelling forms. Morton's cockle, *Laevigatum mortoni*, was conspicuous at all times. This small clam with inflated valves and usually less than an inch in length is of no commercial importance. At the time the boxes were examined finally, there were ten times as many cockles in the box at the east station and they were on the average 50 percent larger than those in the west box. Similar differences have been observed in the growth and set of oysters at these two stations. A hydrographic survey of these two stations by the Oceanographic Department of A & M College of Texas failed to uncover any physical feature in the environment that would account for these differences in the growth of these mollusks.

Finally, on the question of growth, it is possible to demonstrate a differential response due to an entirely different and in this case an explainable cause. Some years ago, we obtained a supply of seed hard clams derived from reciprocal crosses of pairs of the northern and sourthern variety of quahog. These seed clams were in the size range from 1/16 to 1/8th inch when planted and we maintained them successfully for more than 4 years, side by side in protected boxes hung from the laboratory dock. At the end of that time, the stock of pure *M. mercenaria* was on the average 50 percent longer, and the meat yield of shucked clams was nearly double that of the hybrid stock. Whether this was actually a hybrid cross has been questioned, but this is immaterial to the point that they were of controlled parentage. Each group of clams had been derived from separate gene pools and had shown distinctly different growth characteristics in identical biological environments.

Knowing these different growth responses exist, we are impressed with the necessity of knowing the complete background of test animals before hazarding a guess as to the reasons for their observed growth rates in any particular situation. We must know the age of the animal, we must know its genetic background, and we must be assured of the similarity of our test sites before we can suggest that this or that is truly a response to an environmental factor.

REPRODUCTION

The unreliability of random growth-studies for assaying the suitability of the environment to mollusks led us to consider the possibility of using the seasonal gonad cycle in oysters as an index of well-being. Small populations of oysters were kept in protected trays, specimens were sacrificed at monthly intervals, and the activity of the gonads was evaluated on an arbitrary arithmetic scale (Butler, 1949). We were fortunate in having data on the gonad cycle of oysters growing in Chesapeaka Bay, as well as having some Chesapeaka Bay oysters that had been transplanted to Florida, for comparison with the native oyster. In general, the gonad cycle was the same for the three groups with the exception of the timing (Butler, 1955). The Chesapeake Bay oysters, after 1 year of adjustment in Florida waters, started spawning at a temperature level 5°C above that of their parent stock, and reached a stage of mass spawning also at a 5°C higher temperature level. In addition, their spawning period was extended from the usual 3-1/2 to 5 months. Their reproductive activity was indistinguishable from the native Florida oysters. Figure 6 compares the timing and level of gonad activity in the two stocks of oysters at each location.

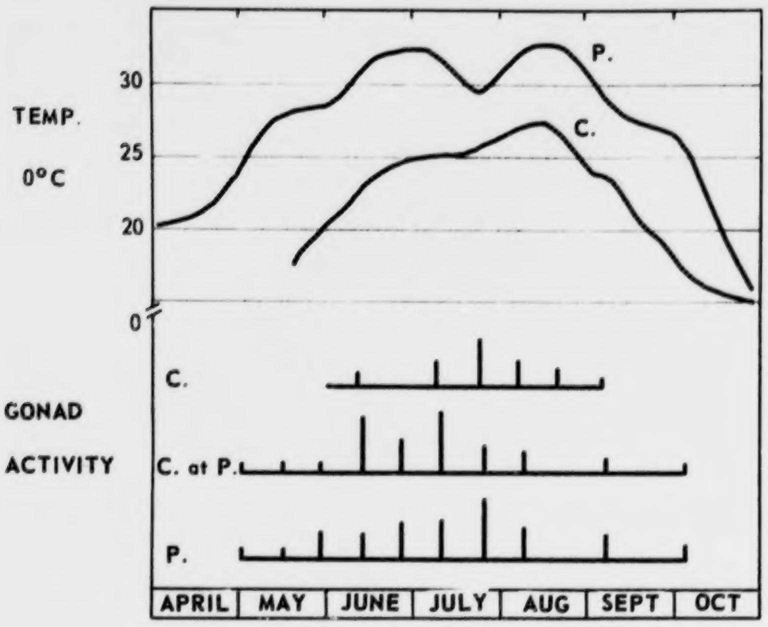

Figure 6. Comparison of reproductive cycle in two stocks of oysters, in which spawning was arbitrarily considered as the maximum level of activity: Chesapeake oysters (C) growing in Chesapeake Bay; Chesapeake oysters (C at P) after growing for two years in Santa Rosa Sound; and (P) Florida native oysters growing in Santa Rosa Sound. The horizontal bars indicate the duration of the spawning periods while the vertical bars, ranging from about 20 to 90 percent, indicate the number of spawners in each sample.

These results suggest that the oyster's reproductive activity is regulated primarily by changing temperature levels rather than a hereditary response to a specific temperature threshold. Examination of our decade of spatfall records in Santa Rosa Sound indicates that spawning is unitiated following a 5°C temperature rise within a 30-day period. The actual onset of spawning has occurred at temperatures ranging from 18 to 27°C, and varies in time from early March to mid-May. In this area at least, the fact that oyster spawning has been initiated tells you essentially nothing about the time of the year or the temperature of the water.

It might be expected that if northern oysters accommodate to the water temperature regimen in the south the reverse might be true. There are numerous citations, however, of the failure of southern animals to spawn when transplanted to more northern latitudes. It is important to note that the response of the oysters here is apparently not to temperature *per se* but to a relative change in temperature. This may account for their failure to spawn in northern waters, although this failure is usually explained on the basis of a particular threshold value, and inferentially, physiological speciation.

Variations in the onset of spatfall from year to year have been mentioned briefly. The timing of the spatfall and the amount of successful set are of particular concern to the commercial oyster grower. They are also of interest to the biologist in considering the relationship of species success and specific factors in the environment. The study of spatfall has been a favorite tool because of the relative ease in obtaining objective data that are susceptible to statistical analysis.

At our laboratory, squires of cement-board offering 100 square centimeters of surface are suspended from the dock and changed at 7-day intervals (Figure 7). The number of sedentary organisms per square centimeter is then calculated by counting a varying percentage of the surface depending on the numbers of organisms present. It is of interest that in more than a decade of such observations, we have always had at least one macroscopic, sedentary animal on the plate by the end of the week (Butler, 1954).

Presumably the amount of spatfall on these plates is one measure of the adult oyster's response to the environment; while this is true, it is by no means the entire story. I am not considering at this point areas in which from time to time the annual set is a complete failure. In Santa Rosa Sound, the annual set is normally so heavy as to be objectionable from the commercial point of view. The density of the spatfall is greatly influenced not only by the amount of spawn produced by the adults but also by events in the pelagic life of the larvae. Their numbers may be affected seriously by sudden hydrographic changes, by disease,

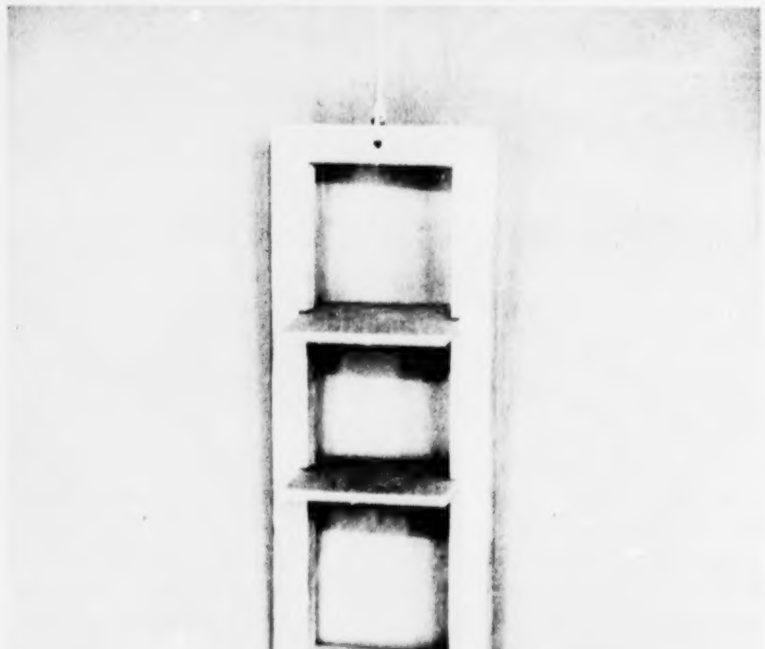

Figure 7. Weighted wooden frame supporting four-inch square cement-board plates used to collect natural oyster set.

r by the relative success of some predator. For hese and other reasons, the record of one year of spatfall in a particular environment may be of little alue.

A summary of a decade of records of spatfall in anta Rosa Sound is shown in Figure 8. A line graph f the averaged data indicates a not unpredictable istribution of the set thruout the summer months, tarting and ending at low levels and with an early ummer peaking of the set. Examination of the records or individual years, however, immediately reveals the rror in this picture. The solid area in each year's ecord indicates the period during which 50 percent f the total set for the year was collected on the lates. This period varied in length from 2 to 6 eeks and more importantly varied from month to onth during the summer, seemingly at random. here was no apparent relationship between the iming and the total set in any particular year. The ccumulated seasonal set varied from 13 to more han 90 spat per square centimeter in the period nder consideration, and in other years, has been early double this amount. Oyster and clam larvae ave relatively short pelagic lives, and Davis (1958) as shown that they are far less tolerant than adults o changing hydrographic conditions. Consequently, hese spatfall data reflect the effect of the environment on the larvae rather than on the mature oyster.

We know too, from long experience, that our estimates of the total weekly set can vary greatly depending on the skill of the technician in handling the cultch plates. Variations in angle of plate, level in the water, intensity of barnacle set and other factors have predictable effects on the successful set of oysters on the plates.

We have had at least one clear demonstration of the drastic effect that environmental factors may have on the intensity of set, and the fact that they exert their influence on the larvae rather than on the adult. On August 30, 1950, this area experienced a severe tropical storm that brought more than 7 inches of rainfall and a storm tide of 5.5 feet. Figure 9 graphically portrays the change in oyster set on artificial cultch during the period of this storm, and in adjacent years when no such storm occurred. It is clear here that the increase in set has been entirely the result of larval survival and not production, since the increase was limited to the period of larval life of the brood and occurred before a new brood could have matured.

Larvae of other sedentary forms as well as the total plankton mass were similarly affected; table 2 shows the relative changes in some setting rates from the period one week before the storm to one week afterwards.

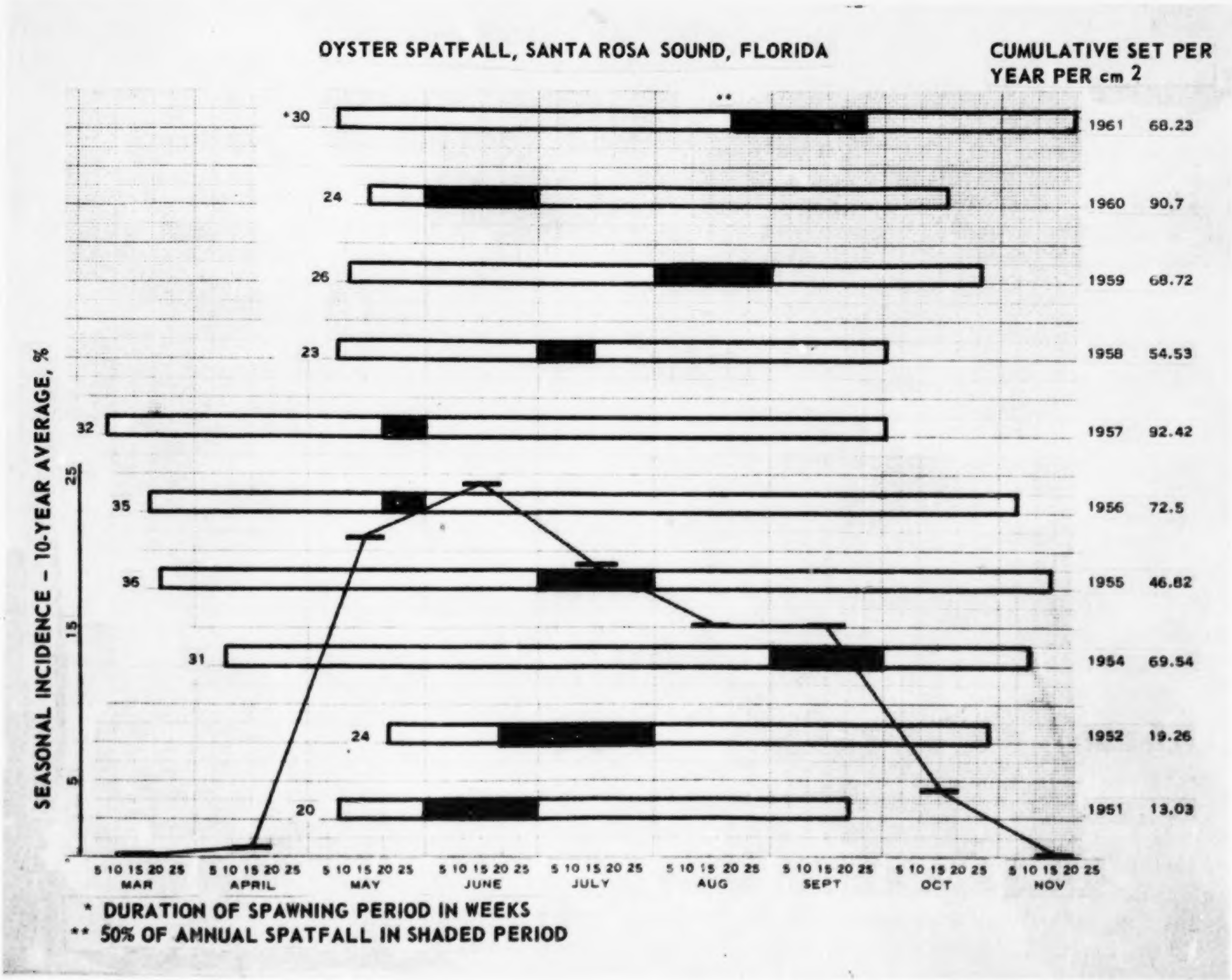

Figure 8. Seasonal and cyclic occurrence of oyster spat on artificial plates, which were changed at seven-day intervals. Data for 1953 incomplete and not included.

Table 2. EFFECT OF STORM ON SETTING OF SEDENTARY ANIMALS

	Incidence of organisms per cm² per week		
	1 week before storm	1 week after storm	
Barnacle	0.3	170.6	568x
Oyster	1.1	73.0	67x
Mussel	0.1	3.2	32x
Bryozoa	0.1	2.1	21x
Plankton M1/100 1	0.9	5.0	5x

In 1953, this area experienced another tropical storm, which was similar in all obvious factors except that the direction of the wind was different and there was no accompanying storm tide. Following this storm there was no observed increase in the set of plankton organisms.

In 1955 tropical storm occurred in the New England area accompanied by high tides; Dr. Loosanoff (1955) reported that more mature oyster larvae were observed than ever before and the set was greater than in the preceding 11 years. The following year, after two tropical storms in New England, he reported that they had experienced the longest setting period in their 20 years of observations (Loosanoff, 1956).

These observations suggest that high waters had leached from the neighboring marshlands some trace elements or simple nutrients that has greatly enriched the estuarine waters. This enrichment caused increased phytoplankton productivity and, in turn, resulted in greater survival of the larval broods.

NUTRITION

The differential in molluscan growth and set on the east and west sides of the laboratory island in Santa Rosa Sound has been a subject for our investigation for some years. We have pursued such environmental factors as temperature, salinity, pH, nutrient salts, dissolved copper, chlorophyll and other plankton pigments, total plankton, turbidity, currents, and others, without identifying any causal relationships. More than a decade ago, the existence in this area of natural substances in sea water responding to chemical tests for carbohydrates was reported by Collier et al. (1950). In the course of an extensive testing program, they found that concentration of this material was positively correlated with the pumping activity of oysters; usually, oysters did not pump during the winter months when carbohydrate levels were less than approximately 4 mg/1, nor in the summer at levels much below 6 mg/1. They postulated a causal relationship (Collier et al., 1953).

We re-examined this situation utilizing the same basic techniques and much of the same equipment,

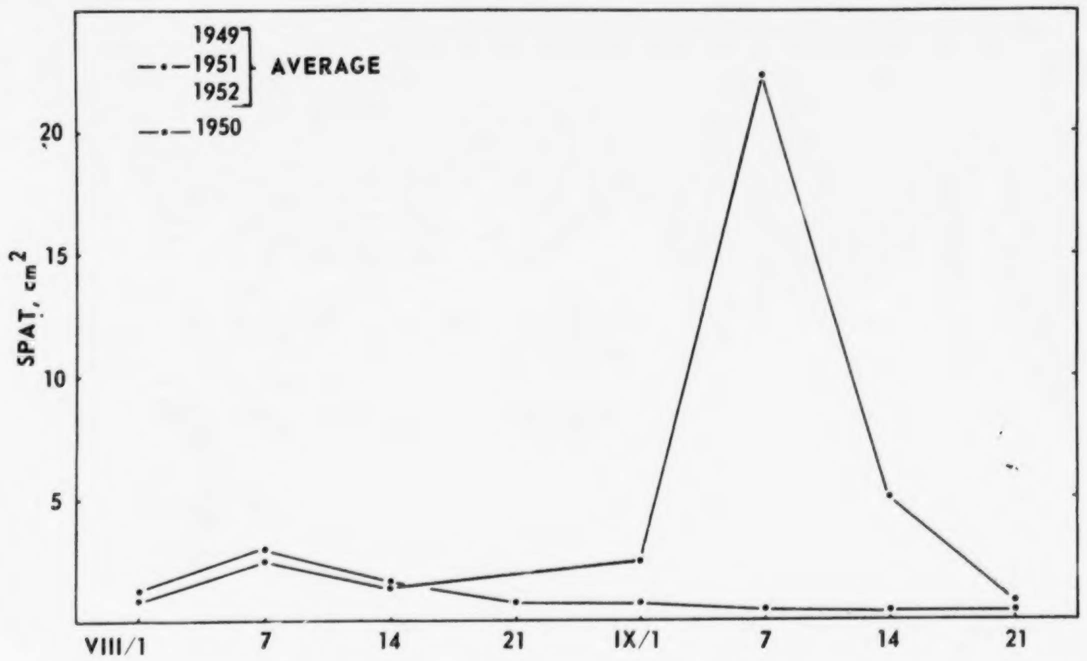

Figure 9. Weekly setting rate of oysters in Santa Rosa Sound in 'average' years, and following the tropical storm of August 30, 1950.

in the hope this might explain our east-west station differential (Butler et al., 1959). We found carbohydrate concentrations surprisingly low as compared to data obtained earlier in the identical geographical area; in fact, considerably lower than what has been reported as minimal threshold values for oyster pumping activity. Consequently, we undertook a series of continuing observations to re-examine the putative relationship between carbohydrate concentration and oyster pumping activity.

Oysters were individually confined by means of a plastic box and rubber dam (Figure 10) so that when the box was attached to the inside of a larger tank the inhalent side of the oyster was exposed to the common water supply, the exhalent side was separated and the water pumped by each oyster could be metered and recorded on a kymograph. Movement of the upper shell was transmitted to the kymograph by means of a thread. Experiments were set up so that the activity patterns of ten oysters were recorded simultaneously and hourly samples of water from the common source were taken for carbohydrate analysis.

Pumping of water by the oyster while not quantitatively related to food intake is ordinarily a prerequisite to feeding. Included here under the general heading of nutrient are various observations on water pumping, shell movement and shell deposition.

Figure 11 illustrates a section of a typical kymograph record of the simultaneous activity of three oysters in the same aquarium over a period of about 3 hours. The top line 'P' of oyster number 1 records the number of times the effluent water bucket was tripped. In this case, pumping appears to be relatively uniform, although the rate varies from about 30 to 50 units per hour or the equivalent of 8 to 12 liters per hour. We are not interested here, however, in the exact quantity of water pumped, but rather in the regularity of the pumping activity. The second line 'S' shows the typically irregular movement of the right valve as the oyster pumps. The vertical lines

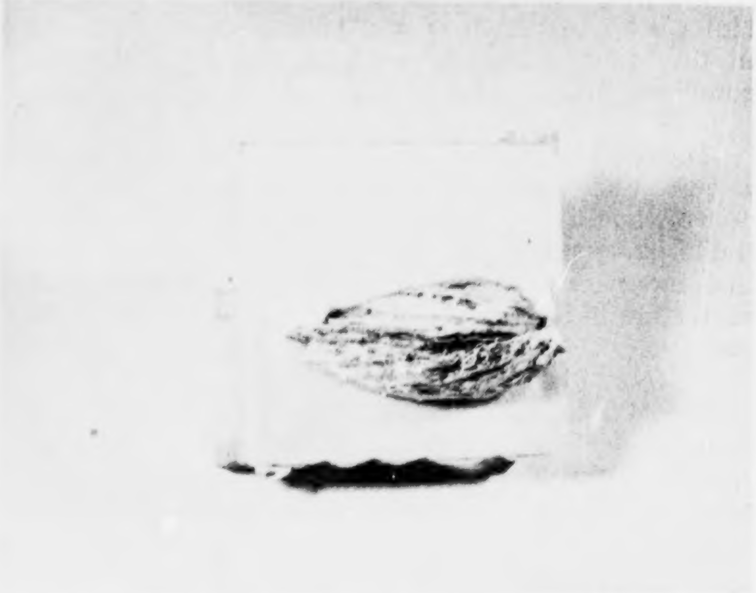

Figure 10. Oyster attached to plastic frame with rubber dam separating inhalent (dorsal) from exhalent side. This makes possible recording the amount of water pumped; thread attached to upper valve transmits shell movement to kymograph.

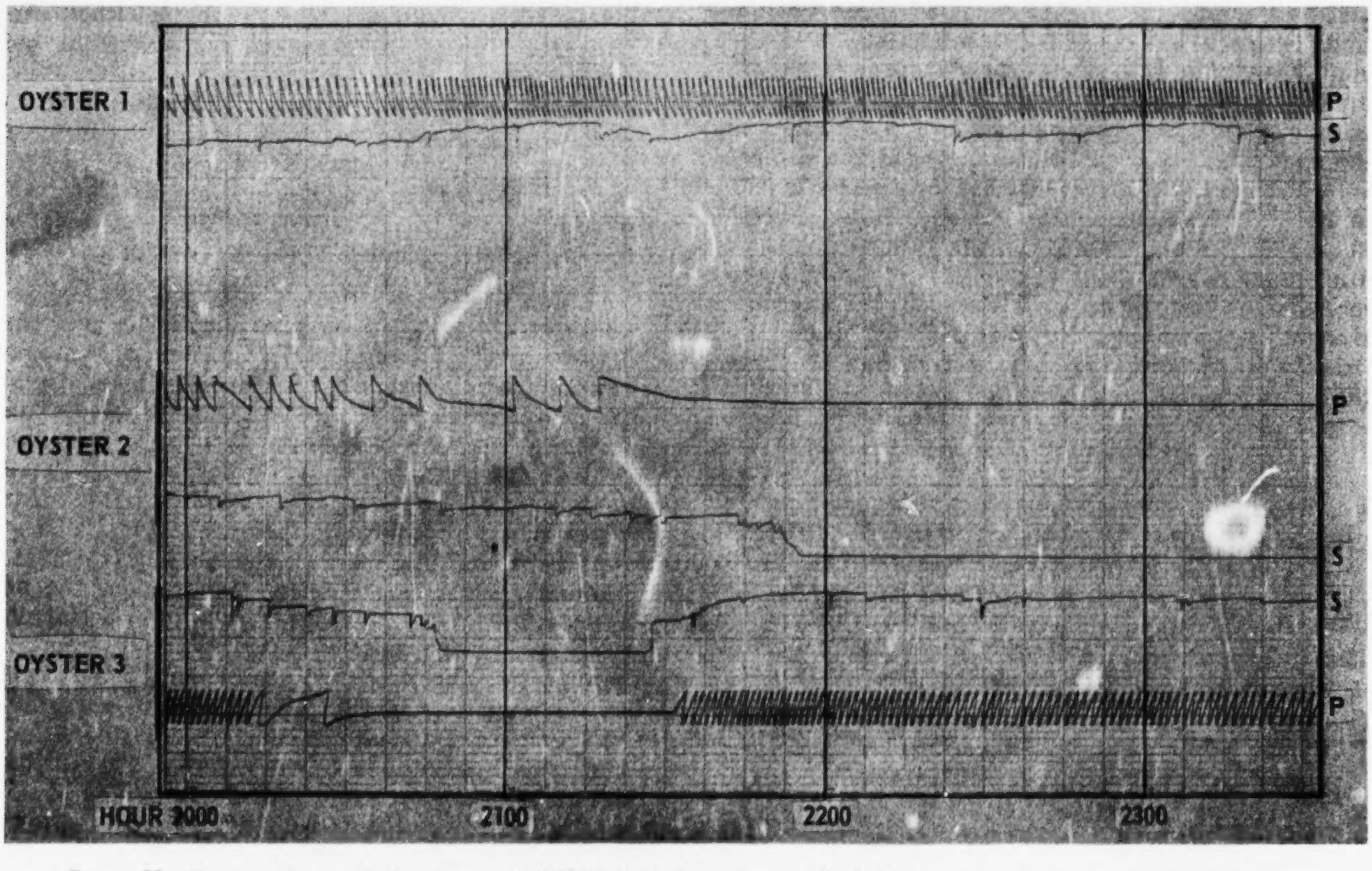

Figure 11. Kymograph record of water pumped (P) and shell movement (S) of three oysters in same aquarium showing individual variations in response to common environment.

indicate an amplitude of 2 to 3 mms in shell movement. In this period the oyster did not close its valves completely.

The record of the second oyster obviously shows a quite different activity. The 'P' line indicates a decreasing rate of activity for the first hour and finally a cessation of pumping. The shell movement line indicates a progressive series of snap closures; each time the valves open somewhat less, and finally, just before 2200, the oyster closed completely.

The third record shows a similar interruption in pumping and shell closure, but after an hour the oyster opened abruptly and continued pumping at its 'normal' rate.

These records illustrate well the individuality of oysters in responding to factors in a common environment. It is apparent that at about 2000 hours there was some external factor, which all three detected but responded to in a different manner.

Our observations in this experiment consisted of searching the kymograph record for each 24-hour period to locate times in which the oysters as a group exhibited normal activity, followed by a progressive slowing down, and then a return to the normal rate of pumping. Only the water samples collected during these stages were analyzed for carbohydrate concentrations. In all, 70 occasions were found that satisfied all of the requirements for our testing conditions. The results of these analyses are shown in Table 3. Carbohydrate concentrations were determined when the oyster was pumping at a normal or regular rate, and then again an hour later when the kymograph record showed that its pumping was slowing down markedly. The data of the initial carbohydrate concentrations were arbitrarily grouped into three ranges. At the lowest initial range of 0-0.9 mg/1 the graph shows that of the 24 occasions when the pumping rate decreased, the carbohydrate concentration decreased 10 times, stayed the same 3 times, and increased 11 times. Data for the other ranges are similar and, in sum, out of the 70 periods, changing carbohydrate concentrations were correlated with decreased pumping negatively 41 percent of the time, positively 40 percent, and showed no change 19 percent of the time.

Table 3. CHANGES IN SEA-WATER 'CARBOHYDRATE' CONCENTRATION WHEN OYSTERS WERE OBSERVED TO BE DECREASING THEIR PUMPING ACTIVITY

Initial concentration of 'carbohydrate' mg/liter	Direction of change and number of observations		
	Decrease	Static	Increase
0.0 - 0.9	10	3	11
1.0 - 1.9	16	10	17
2.0 - 4.3	2	0	1

The second method we used to correlate oyster pumping activity with carbohydrate concentration was to estimate the number of hours of the day when the oyster was actively pumping and compare this with the level of carbohydrate in a composite sample of water for the 24-hour period. The lengths of these periods were tabulated on a percentage basis, and plotted against the carbohydrate levels (Figure 12).

Since in our area oysters may show a differential pumping activity that is apparently correlated with temperature, the data are grouped at two different temperature levels. In the range of 10 to 20°C, i.e., from January through March, daily pumping activity occupied from 65 to 100 percent of the time. Carbohydrate concentrations were all below 4 mg/1. Records of many periods of almost continuous activity were obtained when carbohydrate concentrations were between 0.0 to 0.9 mg/1. A decade earlier, almost no pumping activity was observed at carbohydrate levels below 4 mg/1 in this temperature range.

At temperatures between 20 and 30°C, market size oysters may be expected to show less pumping activity; in this experiment activity ranged from 25 to 95 percent. With minor exceptions, all of this pumping took place at carbohydrate levels below 2.0 mg/1. Collier found at these temperatures it was necessary to have a carbohydrate concentration of approximately 6 mg/1 before pumping was initiated. Figure 12 indicates little if any correlation between increased pumping activity and increased levels of carbohydrate at either temperature range; nor does there appear to be any minimal threshold below which oysters were inactive.

I am reporting this material in some detail because it is important to note that what at one time appeared to be a clear-cut relationship between oyster behavior and a particular parameter of the environment is now open to a different interpretation. This is not to suggest that in the earlier work the activity of oysters was not correlated with carbohydrate concentrations, but rather that at that time oyster activity and carbohydrates were both related to some other and still unknown environmental factor.

Numerous workers have noted the characteristic shell movements of oysters under varying internal and external environmental conditions. The spawning reaction of ripe males, for example, is easily stimulated by appropriate temperature changes and, in turn, the reaction of ripe females to sperm suspension or to temperature is clearly defined (Galtsoff, 1961). A similar group response is illustrated in Figure 13, which excerpts a 5-hour record of two mature oysters in a common aquarium. The shell movement lines indicate a series of snap closures of the shell at approximately hourly intervals. These partial closures occurred at the rate of about two a minute, and had no effect on the pumping rate as seen by the regularity of the pumping record. This response has been noted on numerous occasions and we at first thought it indicated spawning behavior. Later, it was observed during the winter months and commonly a majority of the animals in a community aquarium showed the reaction simultaneously. The reason for this reaction is unknown but it typifies the oysters' sensitivity to apparently subtle factors.

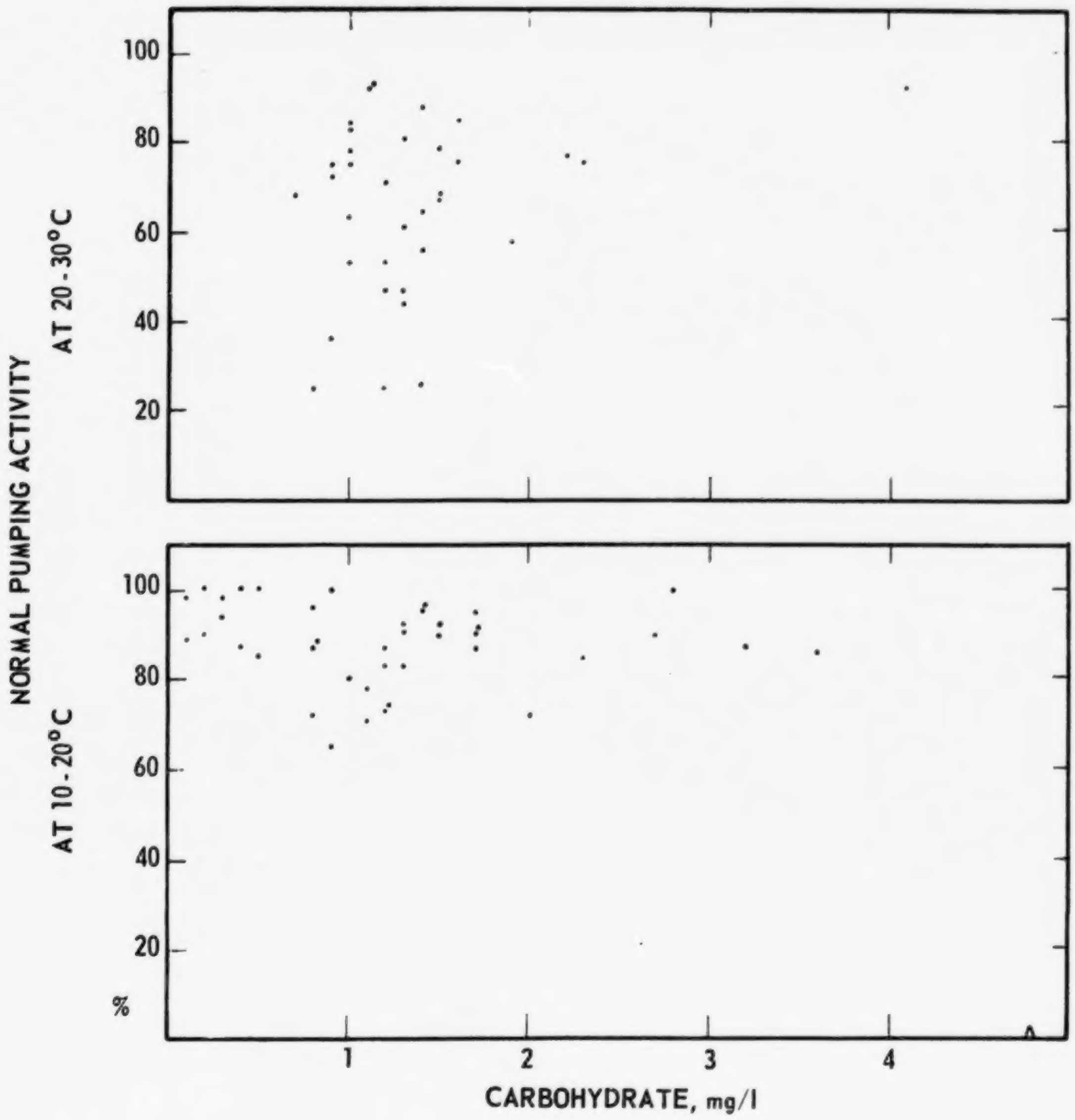

Figure 12. Normal pumping activity, i.e., percent of time oyster was open and pumping, compared to concentration of natural 'carbohydrate' at two temperature ranges. Each dot represents the level of activity of one oyster for one day at the indicated temperature and level of 'carbohydrate'.

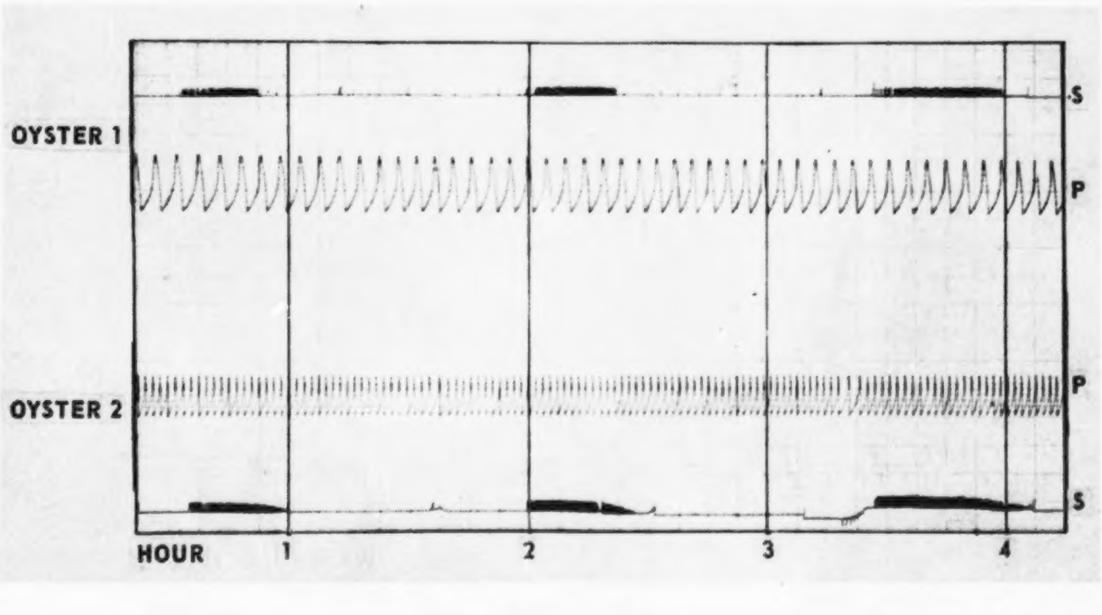

Figure 13. Kymograph record of shell movement (S) and pumping rate (P) of two oysters in a common aquarium. The (S) lines indicate their simultaneous response to some external factor at approximately hourly intervals.

The recording of shell movement and pumping activity of oysters provides a convenient tool for the evaluation of pollutants added experimentally to the environment. In some preliminary work, we found many of the common argricultural pesticides had a marked deleterious action on oysters at concentrations ranging from 0.01 to 1.0 ppm in running sea water (Butler et al., 1960). The number of hours per day that the oyster was open and pumping steadily decreased after a week of continuous exposure to a noxious chemical. For example, after 5 weeks of continuous exposure to Dieldrin at 0.1 ppm the daily activity of exposed oysters was only half that of controls (Figure 14). The graph shows clearly, too, that after removal to clean water there was a fairly rapid recovery.

This reaction of the oyster to its environment is clear-cut and provides useful data. The technique, however, is tedious and time consuming. It is more expedient under the same experimental conditions, simply to measure the rate of deposition of new shell in juvenile oysters. In Figure 14, these data are indicated as percent growth. It is apparent in the graph that there is an immediate decrease in this shell deposition in the experimental oysters, whereas decreases in percent pumping activity of mature oysters were not apparent until after the second week of expousre to the pollutant. The recovery in the rate shell deposition follows the same pattern as the return to normal pumping activity. It is of interest to note the simultaneous response in activity and shell deposition in both experimental and control oysters to the relatively small temperature fluctuation during the terminal 3 weeks.

Linear growth of the oyster does not proceed at a uniform rate under uniform conditions. There is an initial increase in the terminal deposition of shell followed by a period during which this new shell is thickened by internal deposits. During this stage there may be no increase in length. In order to avoid misinterpretation of these length increases, the shell of the oyster is filed until all new or thin shell has been removed; the oyster meats then occupy all of the available space. Under these conditions, the oyster has no alternative in growing except to deposit new shell at the posterior end. Figure 15 shows one of these oysters with the shell newly filed, and deposits of new shell at the end of 2 and 7 days. Under average conditions, shell deposits are made at the rate of approximately 4 millimeters per week in control oysters. The average amount of new shell deposited by oysters under comparable conditions is uniform and statistically valid even for small groups. We are finding it a most useful criterion for evaluating the relative toxicity of a large series of pesticide chemicals and determining at what concentrations these chemicals become physiologically unacceptable to the oyster.

It is apparent from these data on the toxic quality of agricultural pesticides that mollusks exhibit at least three types of reactions. In the first type, as the concentration of the pollutant is decreased, by a factor of ten for example, the rate of shell deposition gradually increases to that of the controls. This may be noted with such chemicals as endrin, aldrin and heptachlor.

In the second type, a given concentration may cause a marked decrease in shell deposition, yet

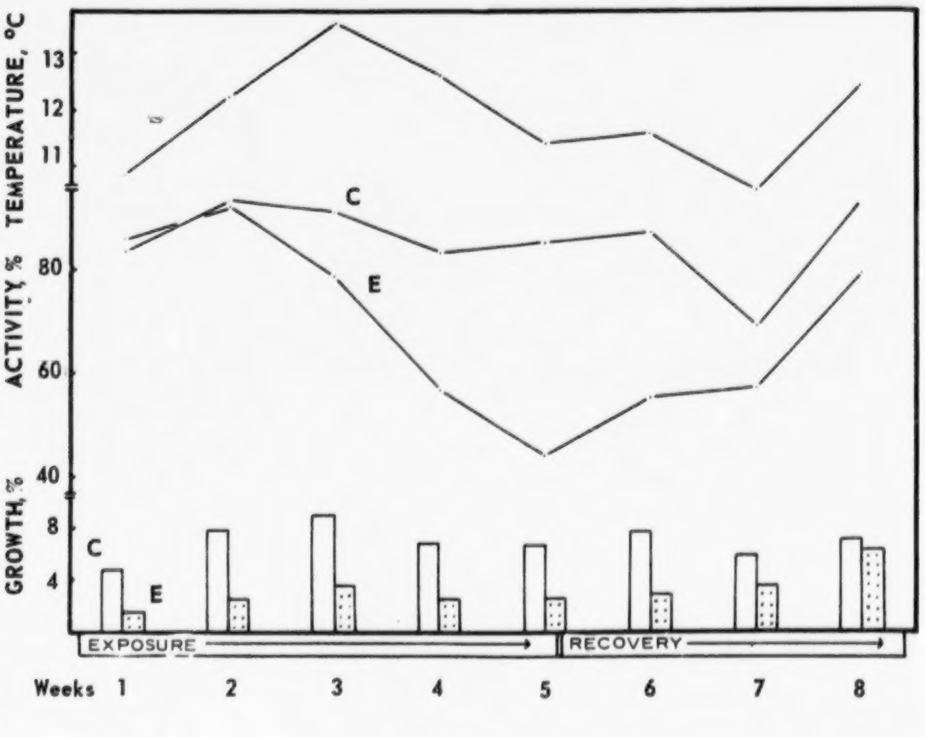

Figure 14. Pumping activity of mature oysters in running sea water exposed to a Dieldrim concentration of 0.1 ppm for 5 weeks, and for the succeeding 3 weeks in unpolluted water. The growth rate of juvenile oysters held in the same tanks (E) is compared to control juveniles (C).

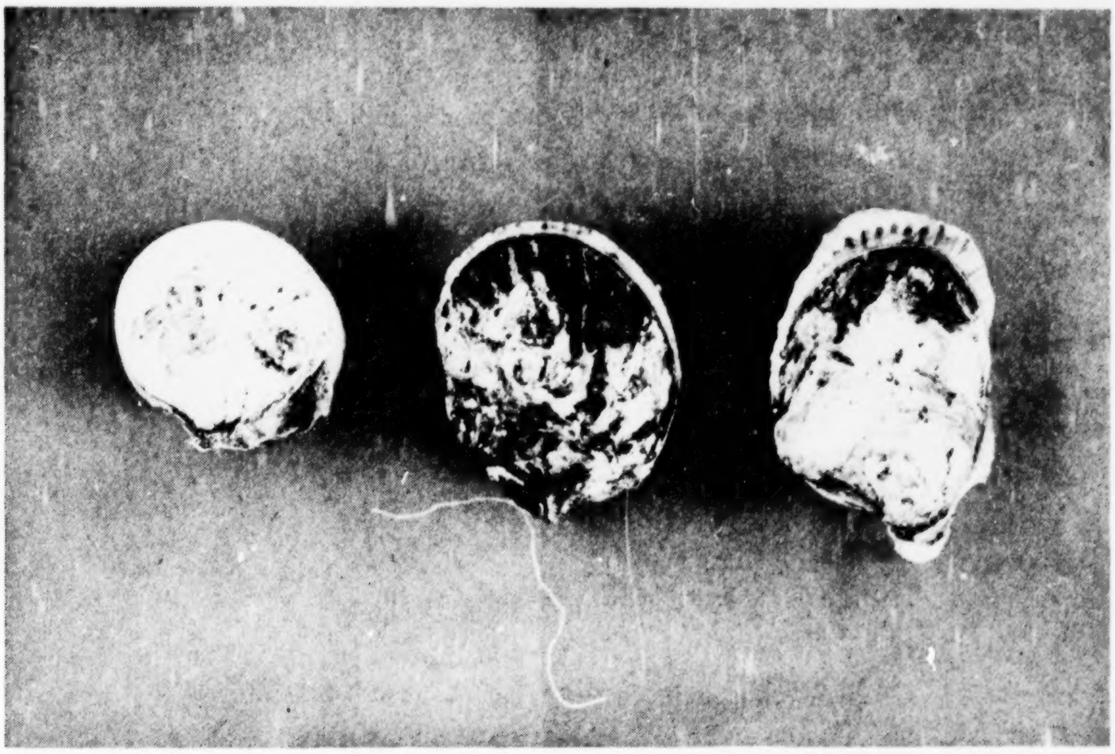

Figure 15. Shell deposition on 1-½-in. oysters. Control on left; after 2 days (middle); and after 7 days (right).

dilution by a factor of ten renders it apparently harmless. It seems that in a wide variety of pesticides, including dieldrin, Kepone, and parathion, there is a fairly sharp threshold level of toxicity.

In this third type, our data are still incomplete. Small hard clams are being exposed over relatively long periods of time to four chemical pollutants at concentrations from 100 to 1000 times more dilute than levels found irritating to small oysters. In the first 3 months, all groups grew as well as controls and showed similar activity and mortalities. In the fourth month, clams exposed to one part per billion of DDT have experienced a sudden mortality of approximately 30 percent. They are sluggish when uncovered and fail to bury themselves in the sand. It is possible in this case, that there has been either an accumulation of the toxicant to lethal levels within the clam or else a loss of resistance on the part of the clam. In either case it indicates the possibility of a build-up, over long periods, of low concentrations of pollutants in an estuary until they finally become disastrous to molluscan populations.

CONCLUSION

A great deal of work remains to be done in identifying the reactions of mollusks to specific natural factors in the environment and to man-made pollutants. It is obvious that variations in environmental parameters may become limiting factors, as they approach extremes. Thus, high or low salinity controls the expansion of oyster populations at either end of the estuary. Similarly, low temperatures suppress spawning the population expansion of the eastern oyster when transplanted to the Pacific coast.

Over a broad intermediate range of environmental conditions, however, the survival of molluscan populations is a result of highly complex and inter-related attitudes toward individual factors. Apparently simple responses, such as growth rates, may be greatly influenced by age, or heredity, or nutrient salts and vitamins naturally present in the environment in only trace amounts.

Although acute toxic levels of natural and man-made pollutants are quite simple to demonstrate, the effects of low levels of toxicants are much more obscure, and disasterous changes in productivity levels might occur without significant mortality. Our ability to delineate the individual factors that are deleterious to estuarine forms will determine in large measure our success in preserving this part of the marine environment, which is so sensitive to pollution. Only by understanding the role of both beneficial and harmful factors will we be able to reach maximum utilization of our estuaries for both biological and industrial purposes.

ACKNOWLEDGEMENT

It is a privilege to acknowledge the assistance and cooperation of the entire laboratory staff whose efforts during the past decade have made this summary possible.

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DISCUSSION

The discussion following the formal presentations immediately centered upon our lack of basic knowledge of the effects of pollution on the estuarine environment. Considerable emphasis was placed upon the need for an investigator to know his experimental animals thoroughly before attempting to interpret the results. The work reported to this time has demonstrated the great diversity of responses that may be expected between or among animal groups and life history stages. Delayed or indirect effects often turn out to be the most important feature uncovered by a given investigation. The subtlety of such responses brought up the objection to "kill - no kill" as parameters in toxicological research. There was general agreement that we should work on the more subtle, physiological effects of sub-lethal toxicity. Comments from the floor supported the participants' views in pointing out the need for basic studies on environmental requirements of larval development since this is often the most sensitive stage in the life history of estuarine animals.

Dr. Carraker concluded the session with a summary of the presentations and suggested that, in selecting or determining the range of parameters which are suitable for the environment in a particular estuary or embayment, the following should be attempted:

1) A systematic study of the dominant species present, so that we have a good taxonomic base upon which to build.

2) A field, ecological-life history and horizontal distribution and density study of the major species.

3) Rear animals in the laboratory under controlled conditions, and subject them to changes in these parameters, one by one, then in combination and then in fluctuation, to try to simulate the complex which exists in the environment.

4) Lastly, to bring to our aid, mathematics and computers to help make a proper interpretation of these conditions.

ENVIRONMENTAL REQUIREMENTS OF AQUATIC INSECTS

T. T. Macan,[*] Chairman

ENVIRONMENTAL REQUIREMENTS OF PLECOPTERA

Arden R. Gaufin[†]

INTRODUCTION

Stoneflies constitute a relatively small order of aquatic insects, with a world fauna of around 1,200 species. Approximately 400 of these have been described for North America. The order is divided into two suborders: the Filipalpia, in which the nymphs and adults of many genera are primarily herbivorous; and the Setipalpia, in which the nymphs are usually carnivores and the adults in most genera do not feed. The world fauna includes nine families (Ricker, 1952), six of which occur in North America.

Stoneflies require moving water for the development of the nymphs, and in most areas the immature stages are passed in streams. In some northern regions such as Sweden the early life history stages of some species have been reported to occur in cold lakes where the shore areas are composed of gravel. (Brinck, 1949).

Although stoneflies constitute one of the smaller orders of insects, they are nevertheless one of the most important groups found in many streams. In mountain streams, they are one of the principal sources of food for trout. Adult members of the genus *Brachyptera* are reported to feed on the tender buds of soft fruit crops in the Pacific Northwest (Newcomer, 1918). Because of their preference for clean, well-aerated water, stoneflies are becoming increasingly important to limnologists as indicators of clean water conditions in pollutional surveys.

REVIEW OF THE LITERATURE

Practically all of the studies to date on the North American stoneflies have dealt with their taxonomy and morphology. The early work of Claassen in New York and Frison in Illinois revealed the diversity of species found in eastern and midwestern United States. More recently the studies of Ricker in Indiana and Canada, Jewett in the Pacific Northwest, Hansen in the New England States, and Gaufin in the Intermountain Region have advanced our knowledge of stoneflies in those sections of North America. Despite the work that has been done to date, the nymphs of over 50 percent of our North American species have not yet been described. This is particularly true of western species.

Research conducted by Hynes (1941), Aubert (1946), and Brinck (1949) in Europe indicates that European stoneflies differ perhaps more in their ecology than they do taxonomically or morphologically. In view of this finding, detailed information as to the habitat requirements and physiological differences of the various species may be essential to stonefly taxonomists if they are to separate the many yet unidentified forms.

Most of the ecological data that have been published concerning North American stoneflies consist of general statements as to habitat preferences. Frison (1935), for example, stated that certain species in Illinois were found only in small streams, while others were found only in large streams. He also indicated that the type of bottom is an important factor governing the concentrations of different species of stonefly nymphs.

Hynes (1941) mentioned five major factors as controlling the distribution of stoneflies in England, namely, the movement of the water, altitude, substratum, drought, and the possibilities of colonization. Brinck (1949) considered the following factors as being most important in determining the distribution of Swedish stoneflies: Movement of the water, substratum, temperature, the amount of certain gases and compounds in the water, and the presence of food. He determined the water velocity, temperature, dissolved oxygen content, and pH in a number of streams and showed how these factors influenced the distribution of certain species of stoneflies. He also classified various stonefly habitats based on different perceptible environmental conditions.

DISCUSSION

CLASSIFICATION OF STONEFLY HABITATS

Several authors, in examining the ecological distribution of stoneflies, have proposed classifications of streams and rivers based on their more obvious physical characteristics (Hynes, 1941; Ricker, 1943;

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and Brinck, 1949). Because the actual effect of many factors controlling the distribution of stoneflies is unknown, a detailed classification of stonefly habitats is impossible, but the classification of Hynes is very useful in pointing out the habitat preferences of stoneflies in England. All of the habitats cited can be found also in various sections of North America.

Hynes classified stonefly habitats into two primary divisions, running water and still water, with a number of subdivisions for each. The running-water habitats include trickles; small stony streams such as are found in hilly and mountainous regions, which vary from 1 to 5 yards in width; stony rivers, also typical of hilly and mountainous regions, which range in size from 10 to 30 or more yards wide; and sluggish rivers. He subdivided still-water habitats into two categories, the stony shores of lakes and the submerged parts of emergent vegetation of mountain pools.

Brinck (1949) in his classification of Swedish streams added the concepts of eutrophication and temperature differences as brought about by differences in latitude and altitude. Hora (1930) discussed in some detail the microhabitats in rapidly running water, which further emphasized the importance of physiographic differences in determining the distribution of stoneflies. The moss found on the top of large stones and boulders, the undersides of large boulders and loose stones, the detritus and sand between the stones and gravel, and pockets of dead leaves and grass all provide habitats preferred by certain species of stoneflies. Moss and algae, for example, provide very suitable conditions for such stoneflies as *Acroneuria pacifica* and *Isoperla fulva* in stony streams of the Rocky Mountains. The undersides of large boulders and loose stones provide the habitat in which larger species such as *Claassenia arctica* and *Pteronarcys californica* usually live and characterize larger streams and rivers. Pockets of dead leaves and grass provide habitats for such species as *Nemoura cinctipes* and *Capnia gracilaria* in small stony streams and trickles of the mountainous western United States.

FACTORS CONTROLLING THE DISTRIBUTION OF STONEFLIES

While a classification of stonefly habitats into the categories given is very useful in explaining the general distribution of stoneflies, detailed studies of the factors controlling their distribution are necessary in order to explain their presence or absence in specific habitats.

Schoenemund (1924 and 1925b) stated that industrial pollution of bodies of fresh water exterminates the stonefly fauna, and indicated that the occurrence of some species in certain bodies of water is influenced by the oxygen content, the substratum, and the rate of flow of water. Several authors in Europe and Claassen and Frison in the United States described in general terms the habitats of various species and genera, but the first real attempt to define factors controlling distribution of stonefly nymphs was made by Hynes within the Lake District in England in 1941.

In "The Taxonomy and Ecology of the Nymphs of British Plecoptera" he lists five major factors as controlling the distribution of stonefly nymphs:

(1) The movement of the water;
(2) the altitude, which is probably a function of temperature;
(3) the substratum, which is to a certain extent controlled by the movement of the water;
(4) drought and its relation to the length of nymphal life of the species and the emergence period of the adults;
(5) the proximity of habitats in which stoneflies are abundant; this factor he refers to as colonization.

In addition to these factors Brinck (1949), in his comprehensive "Studies on Swedish Stoneflies," considers of primary importance such factors as water temperature, the amount of certain gases and compounds in the water, and the presence of food. The author has found that all of these factors are also very important in determining the distribution of stoneflies in streams of the Intermountain Area of western United States, but a factor of equal importance is man's activities. In the United States, pollution, construction of reservoirs, dredging, and water diversion perhaps influence the distribution of stoneflies more than all other factors combined.

Each of these factors is discussed separately in the remaining portion of this paper. In addition, the results of experimental laboratory studies conducted in the last 4 years by the author and his graduate students are briefly considered where applicable.

WATER MOVEMENT

The movement of water is perhaps the most important factor controlling the distribution of stonefly nymphs in that it controls the oxygen supply of the water and to a certain extent the temperature, substratum, and food. Stoneflies are very rarely found in stagnant water and thus are confined almost entirely to moving streams or wave-swept shores.

The oxygen content of the water during nymphal development is an important factor in the distribution of stoneflies. Under normal conditions in almost all streams or lakes where stoneflies occur the water is saturated with oxygen. Certain species, however, such as *Nemoura cinerea* and *Taeniopteryx nebulosa* have been reported by Brinck (1949) as being present in sluggish streams and rivers in Sweden in which the oxygen concentration dropped to 42 percent saturation. The author also encountered two species of stoneflies in streams in Ohio living under similar conditions of oxygen reduction. *Taeniopteryx maura* was collected in large numbers from the Great Miami River above Dayton, Ohio, in sections of the stream where the dissolved-oxygen concentration dropped to 4 ppm at night. In this connection, an interesting but unexplained phenomenon was discovered involving a number of the nymphs. Most of the nymphs taken in the most stagnant sections of the stream where little movement occurred possessed protruding anal gills. In a species in which no such gills are supposed to occur this was indeed surprising. It is surmised that the populations with anal gills may have been better adapted to obtain oxygen than normal specimens of this species. This supposition is partly substantiated by our finding another species, *Perlinella drymo*, in large numbers in a quite unusual and unexpected habitat. This species is comparatively rare and to the author's knowledge has been collected only in limited numbers from riffles in fast-flowing streams of eastern North America. A considerable number of specimens was taken from very slow-moving water from beneath undercut, grass-covered banks from Indian Creek near Miamitown, Ohio. They were found in association with burrowing mayflies, *Hexagenia* sp., and damsel fly nymphs, *Agrion* sp. and *Argia* sp. The thoracic gills of this species of stonefly are very conspicuous and are perhaps longer and more copious than in any other species of North American Plecoptera.

Pollution with organic wastes will often cause a considerable deficiency of oxygen in streams. Brinck (1949) reported that polluted sections of streams in Sweden with an oxygen content below 40 percent saturation had an insignificant stonefly fauna or none. This may have been due, however, not only to the lowered oxygen content but also to the poisonous substances such as hydrogen sulfide produced by the breaking down of the sewage. In fact, laboratory studies being conducted by the author cast some doubt on the supposedly high oxygen requirements of stoneflies. Two species, *Acroneuria pacifica*, and *Pteronarcys californica*, have been maintained in water with an oxygen concentration as low as 1 ppm for 7 days at temperatures of 50° to 51° F with no mortality occurring. One nymph of *Acroneuria pacifica* exposed to this low concentration later emerged as an adult when it was restored to normal conditions.

In addition to its role in oxygenation of the water, water movement is important in several other ways. In slow-moving streams, emergent vegetation can develop, which provides a habitat in which some species of *Nemoura* can live. Fast-flowing water is generally without rooted vegetation, and often deposited dead leaves and detritus are lacking. Species of stoneflies dependent on these materials for food are thus eliminated from streams where the current may be strong. On the other hand the slender nymphs of such genera as *Leuctra* and *Alloperla* are able to live in rapid currents because they are able to work their way down among the stones and gravel and find detritus that has been deposited there. Omnivorous species such as *Isoperla fulva*, *Isoperla patricia*, and *Isoperla mormona* are very little affected by strong currents, and thrive in fast-moving streams because of the algae that grow under such conditions.

ALTITUDE AND TEMPERATURE

Extensive collections of stoneflies taken from streams in the Intermountain Area of the United States during the last 15 years at altitudes ranging from 3,000 to 12,000 feet clearly indicate a distributional pattern that correlates closely with altitude. Because the effect of altitude is primarily one of temperature, these two factors are considered together. The distribution of stoneflies in the Provo River, Utah, a stream that has been intensively studied by the author since 1946, illustrates very well the effects of altitude and temperature on the species present. The river is a typical, swift-flowing, well-aerated mountain stream. It has its origin in the high Uinta Mountains of northeastern Utah and runs southwesterly for 72 miles to its outlet at Utah Lake. The upper section of the river is characterized in many places by falls and cascades, but midway in its course this turbulence is checked by the presence of Deer Creek Reservoir, a storage basin 8 miles long. As the river enters Utah Valley the rate of flow is slowed markedly and the water temperature is notably higher than in the upper portions of the stream. The river originates at an altitude of approximately 10,000 feet and empties into Utah Lake at an altitude of 4,400 feet.

Six species of *Alloperla* have been collected from the river, but none at an altitude below 5,300 feet. Two species, *Alloperla borealis* and *Alloperla pintada*, were taken only at altitudes above 5,900 feet and *Utaperla sopladora* only above 6,400 feet. On the other hand, *Isoperla mormona* and *Pteronarcys californica* were found only at altitudes below 5,900 feet, but closely related species, *Isoperla fulva* and *Pteronarcella badia*, were found throughout the river. In a similar study being conducted on the Colorado River only two species of *Isoperla* have been collected in the lower section below 4,000 feet, whereas various species of *Alloperla*, *Leuctra*, and *Capnia* occur above 10,000 feet. In the lower, turbid Colorado near Moab, Utah, the water temperature often exceeds 70°F in the summer. In the upper Colorado and its tributaries, water temperatures rarely exceed 50°F. The extreme turbidity of the lower Colorado, however, undoubtedly is more restrictive to stoneflies than the altitude or temperatures are.

THE SUBSTRATUM

The substratum of both lakes and streams is very important to stonefly nymphs. Pure sandy bottoms or a muddy bottom are very seldom if ever inhabited by stoneflies. On stable substrata consisting of large stones and boulders, moss and algae often develop on tops of the stones and form a different microhabitat than is found among stones and loose gravel. Nymphs adapted for clinging, as exemplified by several species of *Isoperla* and *Nemoura*, are typical of the former habitat; small slender species belonging to the genera *Alloperla* and *Leuctra* are more commonly found among stones and loose gravel in an unstable substratum. Some carnivorous forms such as *Acroneuria pacifica* on the other hand have long legs and strong claws and occur in either type of habitat.

DROUGHT

In the arid western United States, many streams dry up during the summer and consequently support a very poor stonefly population or none at all. Even in the high Rocky Mountains, where the period of drought is limited, intermittent streams support few stoneflies. By comparison, however, in the eastern and midwestern states, stoneflies can be found abundantly in many streams that dry up regularly each year. The author encountered many small intermittent streams in Ohio that support an abundant and varied stonefly population during the winter and spring months. The adults of some common species emerge in the winter or spring and have a 1-year life cycle. Species occurring regularly in such streams are *Allocapnia forbesi*, *Allocapnia vivipara*, and *Nemoura nigritta*. The nymphus of these species are very small or the eggs have not hatched when the streams in which they occur are dry, which enables these forms to withstand drought conditions. The younger nymphs work their way down into the substratum to available moisture and thus escape disiccation. Species having 2- or 3-year life cycles, such as *Pteronorcys californica* and *Acroneuria pacifica* cannot avoid disiccation in a similar manner and are consequently unable to live in streams that dry up regularly.

The lack of vegetative cover may very well account for the inability of stonefly nymphs to last through the hot summer months in dry, unshaded streambeds in the arid west. The shade afforded dry streambeds in the eastern states by a forest canopy keeps the bottom moist and prevents the eggs or small nymphs of the stoneflies from becoming completely desiccated.

COLONIZATION

Most adult stoneflies stay close to the water in which they lived as nymphs, hiding under stones or in nearby vegetation. A number of species are brachypterous such as *Capnia columbiana* and *Isogenus parallela*, and very seldom can be found very far from the water's edge. Some of the smaller *Isoperla* and *Alloperla* can fly readily though slowly, and will fly to other bodies of water than that from which they emerged. Thus the adults may fly to a new habitat and lay their eggs, and the nymphs may grow and reach maturity, and colonize a new area. While many of the smaller species of stoneflies undoubtedly increase their distribution in this manner, one of the primary factors limiting the distribution of many of the larger species is their relatively poor ability to fly.

EUTROPHICATION AND AVAILABILITY OF FOOD

The kind of food and its availability are ecological factors of great importance that determine the distribution of many species of stoneflies. As already indicated, large moss- or algae-covered boulders in swift streams may provide food for some species of stoneflies that can withstand a rapid current, whereas the detritus accumulating among loose stones and gravel may be an essential source of food for other forms. In the Rocky Mountains there are many clear, cold, but intermittent streams that would seem to provide ideal conditions for stoneflies but that are literally biological deserts. Many such streams constitute roaring torrents in the spring and early summer, being fed by melting snow. In the late summer and autumn they may dry up completely. Most such streams contain so little nutrient material as to be completely unable to support enough plant or animal life to serve as food for colonizing stoneflies.

In some of these snow-fed trickles or streams that are permanent enough to establish a scanty diatom cover on the stream bottom, populations of *Alloperla*, *Leuctra*, *Capnia*, and *Nemoura* may develop and thrive. By contrast, streams that are more productive or eutrophic may produce far more food material, but provide a much less suitable environment, resulting in a less varied stonefly population. For example, the upper Yampa River near its headwaters at Yampa, Colorado, is a highly productive stream with a very profuse growth of algae covering the rocks. Caddis fly larvae, *Hydropsyche* spp., and mayfly nymphs, *Ephemerella grandis*, abound. Stoneflies are common, but the variety is not nearly as great as in a nearby soft-water stream located in an adjacent drainage. In the Yampa, *Alloperla signata* and several species of *Isoperla* are to be found commonly in May and June, whereas in nearby Toponas Creek *Leuctra* spp., *Nemoura* spp., and *Alloperla pallidula* dominate the aquatic insect population.

POLLUTION

Because of the variety of pollutants that are being released into our streams and lakes, only brief consideration can be given to their effect on stoneflies. Organic wastes can completely eliminate most species of stoneflies through oxygen depletion, by the production of toxic products of decomposition, or by changing the nature of the substratum. A dense growth of sewage fungus on the bottom of a stream is an unsuitable habitat for most stonefly nymphs. Most industrial wastes if present in sufficient concentration are likely to be toxic. Hot water from cooling processes and electrical plants, in sufficient quantities, can raise the temperature of a receiving stream above the tolerance levels of most species of stonefly nymphs.

In bioassays being conducted at the University of Utah with stoneflies as test organisms, a number of insecticides are proving to be very toxic to *Acroneuria pacifica* and *Pteronarcys californica*. Ten different insecticides have been tested to date, representing both the chlorinated hydrocarbon and the organic phosphate groups. When the two species have been compared, the resistance of *Pteronarcys californica* has been much greater than that of the *Acroneuria pacifica*. Large specimens of both species have been more resistant to poisoning than smaller ones. This resistance might be a surface-volume relationship, or the smaller nymphs might be more susceptible due to the inhibition of critical enzymes that aid in the molting process. Substances, such as neotinin, which are known to be in higher concentrations in smaller nymphs than in larger ones, might be inhibited by these pesticides.

Of the insecticides tested, DDT and aldrin were the least toxic to both species. DDT is nontoxic to both species at a 0.1 ppm concentration. Aldrin was not toxic to *P. californica* at 0.1 ppm, but killed 70 percent of specimens of *A. pacifica* after 96 hours. Ninety-six-hour TL_m values for DDT for the two species were 1.8 ppm and 0.32 ppm, respectively.

Endrin was the most toxic insecticide tested, being lethal to 100 percent of the specimens of *A. pacifica* after 24 hours of exposure to a 0.1 ppm concentration. The same mortality occurred with *P. californica*, but only after 72 hours of exposure. Ninety-six-hour TL_m values for the two species were 0.00032 ppm for *A. pacifica* and 0.0024 ppm for *P. californica*.

SUMMARY

While stoneflies constitute one of the smaller orders of insects, they are nevertheless one of the most important groups found in many streams. In mountain streams, they are one of the principal sources of food for trout. Brinck (1949) reported that streams in Sweden with dissolved-oxygen concentrations below 40 percent saturation had no stoneflies. Because of their preference for clean, well-aerated water, they are becoming increasingly important to limnologists as indicators of clean water conditions in pollutional surveys.

Practically all of the work that has been done to date on the North American stoneflies has dealt with their taxonomy and morphology. The early work of Claassen in New York and Frison in Illinois revealed the diversity of species found in eastern and midwestern United States. More recently the studies of Ricker in Canada, Jewett in the Pacific Northwest, Hansen in the New England States, and Gaufin in the Intermountain Region have advanced our knowledge of stoneflies in those sections of North America. Despite the work that has been done to date the nymphs of over 50 percent of our North American species have not yet been described. This is particularly true of our western species.

Research conducted by Hynes (1941), Aubert (1946), and Brinck (1949) in Europe indicates that European stoneflies differ perhaps more in their ecology than they do taxonomically or morphologically. In view of this finding, detailed information about the habitat requirements and physiological differences of the various species may be quite essential to stonefly taxonomists if they are to separate the many yet unidentified forms.

Most of the ecological data that have been published concerning both European and North American stoneflies consist of general statements on habitat preferences. Frison (1935), for example, stated that certain species in Illinois were found only in small streams, while others were found only in large streams. He also indicated that the type of bottom is an important factor governing the concentrations of different species of stonefly nymphs.

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The author has found that such factors as temperature, water velocity, bottom type, chemical composition of the water, and abundance of food are very important in determining the distribution of stoneflies in streams of the Intermountain area. The environmental requirements of stoneflies may be determined in artificial streams more precisely than can be done under field conditions. The objectives of laboratory studies conducted during the past three years have been to determine the effects of low dissolved-oxygen concentrations at various temperatures and stream velocities on the gross activity of stoneflies, and to determine the minimum dissolved-oxygen concentrations at which exposure for a prolonged period of time could be endured without lethal effects.

Optimal water temperatures for the species of stoneflies tested to date have been found to be between 50° and 60° F. In this temperature range two species of stoneflies have been exposed to oxygen concentrations as low as 1 ppm for 7 days with no mortality occurring. Sensitivity to low dissolved-oxygen concentrations increased rapidly with an increase of temperature above 60° F.

In the United States, pollution, construction of reservoirs, dredging, and water diversion are doing more to influence the distribution of stoneflies than all other factors combined. Concentrations of insecticides, such as endrin, which may accidentally pollute our streams and lakes, can be toxic to stonefly nymphs at the unbelievably low concentration of only 0.00032 ppm.

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ENVIRONMENTAL REQUIREMENTS OF EPHEMEROPTERA

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Mayflies, an ancient group geologically, have retained sufficient adaptability to continue their occupying almost all fresh-water situations, both lotic and lenitic, from Arctic to Tropics, and from sea level to above timberline. Some species occur over a wide geographic range and may complete their life cycle in 1 year in the warmer part of the range but require 2 years in the colder portions.

Our knowledge of enviromental requirements of individual species is sketchy in the extreme. For many of our North American species, life cycles are unknown -- immature stages undescribed and total span of emergence periods unrecorded -- and only a handful of species has been the subject of detailed study.

Ephemeroptera, and in fact most of the so-called lower orders of aquatics, have received little attention in North America from professional entomologists -- little attention, that is, compared with most of the terrestrial orders that attracted interest from the first because of the damage they did to man's interests or because of their beauty.

During the early years when North American entomology was, quite understandably, dominated by purely descriptive taxonomic work, those concerned with the lower aquatic orders in general and the Ephemeroptera in particular, could be counted on one hand with fingers to spare -- Hagen, imported from Germany to the Museum of Comparative Zoology; Walsh of the University of Illinois, born and educated in England; and Thomas Say, often called the Father of American Entomology. Really significant inroads on the mayfly fauna of this continent were the achievement of workers still alive or only recently deceased-- James G. Needham and his associates and students at Cornell, and, in Canada, J. McDunnough on taxonomy and a distinguished succession of workers at the Ontario Fisheries Research Laboratory.

As one reviews historical developments, it is hard to avoid the conclusion that entomology, as a profession, has steadfastly refused to recognize either the economic importance or general scientific interest of the so-called lower aquatic orders. The vacuum this neglect created has invited the attention of other disciplines. So it is that much of the work on the biology of these orders during the last 3 or 4 decades has been performed by scientists who would reluctantly, if at all, identify themselves as entomologists. Fishery scientists, limnologists, and microbiologists have contributed the greatest share of our knowledge to date on the life histories, distribution, and ecology of the Ephemeroptera and their ecological associates.

The attempt to provide a scientifically sound rationale for management of fresh-water sport fisheries provided the initial impetus for such work. The stream surveys begun by the New York State Conservation Department in the 1920's included sampling of aquatic invertebrates as a routine procedure; this practice led to the incorporation of the fish food fauna as a factor in determining the "grade" of a stream for fish management purposes. Rough evaluation of the place of various aquatic insects in the fish food cycle and the relative abundance in different stream situations yielded data that fishery biologists found useful. When the Michigan Department of Conservation pioneered the concept of trout stream improvement in the early 1930's, top consideration was given to the design and installation of environment-modifying structures that would increase aquatic insect production as well as create other environmental conditions favorable to trout. It is timely to mention that this important pioneering work took shape at the hands of our host, Dr. Clarence Tarzwell.

Because so much of the earlier work on mayfly biology was associated with fish management, activity was greatest in waters of quality adequate to support fisheries of significant value. Pollution due to human activity was rarely a factor, and even today we have much more knowledge of mayfly distribution in "normal" environment than (when it) is affected by allochthonous chemical factors.

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The respective roles of water temperature, depth, and velocity; composition of the substrate; aquatic vegetation and drift materials; and characteristics of the adjacent streambank or lake shoreline, or both have received frequent attention (see list of references). Despite the usual overlapping of range and the adventitious occurrence of off-site species due to drifting, the experienced collector working in a familiar region can usually predict with considerable accuracy the species composition of the mayfly fauna in any given site from information on the foregoing factors. Several comprehensive studies of mayfly "associations" in lakes (Lyman, 1956) and streams (Ricker, 1934; Sprules, 1947) have been made in attempts to devise workable systems for ecological classification.

Several book-length treatments of mayfly faunas of states, covering Florida (Berner, 1950), Illinois (Burks, 1953), California (Day, 1956), and Michigan (Leonard and Leonard, 1962), have appeared within the past 15 years in addition to the continent-wide handbook of Needham, Traver, and Hsu (1935) and a checklist of the fauna for North America north of Mexico (Edmunds, 1957a). The list of references accompanying this paper is an attempt to sample the rather voluminous literature containing contributions to our knowledge of North American mayfly biology, ecology, and economic importance; it excludes titles largely or wholly taxonomic in nature.

Significantly, only a few of the cited titles deal with the effect of pollution on Ephemeroptera at the specific level, which, it is becoming clear, is the only meaningful level. Reports of death or survival of these insects under exposure to a variety of chemical and physical insults are all too commonly based on identifications carried no further than order or family; and even generic identifications can be misleading. Refreshing exceptions are the work of Gaufin and Tarzwell (1952) and a few isolated but important observations such as that by Britt (1955a), who offered one of the first and certainly the most dramatic of the warnings that eutrophication of Lake Erie had already reached a critical point. Britt found that the unexpected development of thermal stratification in the shallow waters of Lake Erie in the vicinity of the Bass Islands between September 1 and 4, 1953, caused dissolved-oxygen concentrations to drop as low as 0.70 ppm near the bottom, with the result that on September 5 he was able to count as many as 465 dead *Hexagenia limbata* and *H. rigida* nymphs per square meter. He ruled out high water temperatures as a factor by noting that nymphs of these species survived "for days" in shallow laboratory trays at 29°C with adequate aeration.

Sloan (1956), who plotted distribution of mayflies in two springfed Florida streams according to dissolved oxygen, chlorides, current velocity, pH, and temperature, noted that the faunas of "springhead" areas resembled those of organically polluted waters probably because of low oxygen levels in both situations.

In Michigan we have frequently observed nymphs of *H. limbata* and *Ephemera simulans* swimming up to holes chopped in lake ice and undergoing winterkill. And during summer droughts we have observed distress and death of nymphs of *H. limbata, H. recurvata,* and *H. atrocaudata* in beaver dams apparently incident on cessation of inflow and consequent reduction in area and volume.

Attention should be called to the finding of Benson (1955) that mayfly nymphs may be embedded in anchor ice and, apparently, transported alive for considerable distances when the ice lifts.

Too often, when new and unexpected pollution enters a body of water, the reaction of the mayfly population cannot be determined because no prior survey information is available. Fetterolf (1962) has noted two instances where release of plating wastes resulted in complete fish kills though at least some mayflies survived. In the Chippewa River near Mt. Pleasant, Michigan, in October 1957, there was a complete fish kill involving minnows, suckers, rock bass, bluegills, crappie, and northern pike. However, nymphs of *H. limbata* and *E. simulans* survived concentrations of 0.20 to 0.30 ppm of cyanide for 1 to 2 hours, and unidentified species of *Caenis, Tricorythodes,* and *Stenonema* survived 0.10 to 0.15 ppm of cyanide for 2 to 3 hours. Fetterolf found in a similar episode in Hayworth Creek near St. Johns, Michigan, in April 1958, a complete fish kill involving darters, logperch, common shiners, madtoms, suckers, rock bass, and smallmouth bass. Here, *Caenis* sp. survived. Concentrations of at least 2.5 to 3.0 ppm of cyanide were present for a period of 1 hour, and concentrations of 0.4 ppm of cyanide and 0.3 ppm of copper were found 4 hours after the initial slug had passed. *Stenonema tripunctatum, S. ares*,, and a *Stenonema* of the interpunctatum group, as well as two species of *Leptophlebia* (presumably *cupida* and *nebulosa)*, survived initial concentrations of at least 2.0 ppm of cyanide and concentrations of 0.3 ppm of cyanide and 0.1 ppm of copper found 4 hours later. *Paraleptophlebia guttata, Baetis brunneicolor, S. fuscum,* and *S. vicarium* survived where the initial concentration of cyanide was 2.0 ppm and that of copper 1.1 ppm, and where a concentration of over 1.0 ppm of cyanide was present for more than 5 hours. One recently killed *P. mollis* was found at this station, and none were found alive.

Figure 1 shows graphically the effect of pollution sources and of a primary treatment plant on nutrient quality, primary production, and relative abundance of mayfly nymphs in a 35-mile stretch of the Red Cedar River upstream from East Lansing, Michigan, that has been studied for several years by Prof. R. C. Ball and his group at Michigan State University. It should be noted that fish are (totally) absent from the stream for 5 or 6 miles downstream from the discharge point of the plating plant effluent, shown 33 miles above the river mouth. Baetine nymphs make their appearance in small numbers (0.9 per square meter) about 2 miles below the plating plant pollution source but ephemerids (principally *Ephemera simulans)* are absent for 12 miles, suddenly appearing in large numbers (188 per square meter). Baetines appear little affected by the treated disposal plant

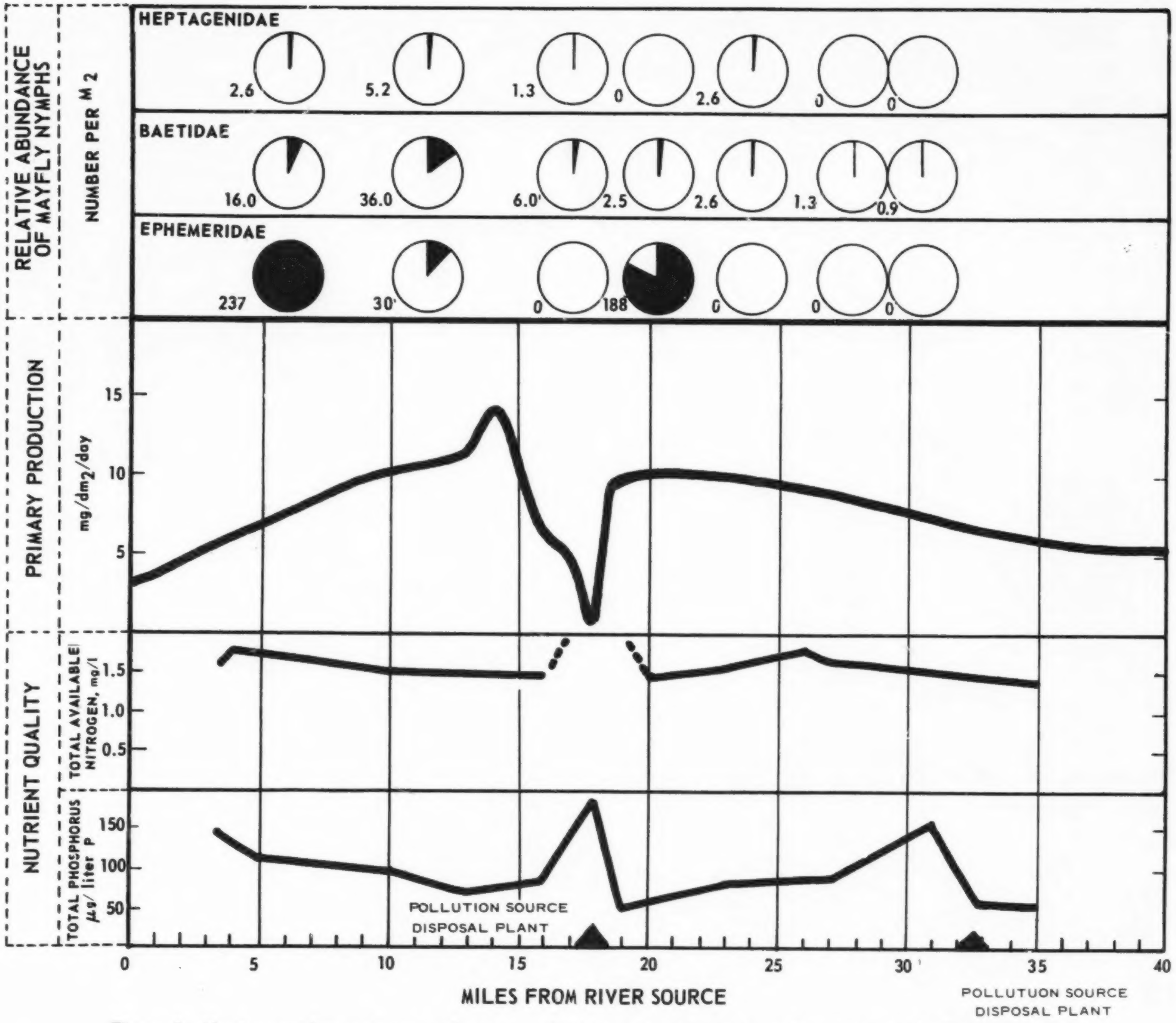

Figure 1. Nutrient quality, primary production, and relative abundance of mayfly nymphs in the Red Cedar River, Ingham and Livingston Counties, Michigan.

effluent discharged 18 miles above the river mouth, averaging 6 per square meter throughout a 2-mile sampling stretch below the disposal plant discharge point. Ephemerids reappear 6 miles downstream from the disposal plant discharge point but become nearly 8 times as numerous 11 miles downstream.

One of the most exasperating deficiencies in our knowledge of mayfly ecology is that concerned with causes underlying wide variation in production levels in natural waters. In "natural," pollution-free streams, it is generally found that the numbers of individuals and volume of nymphs (but not necessarily numbers of species) are lowest in sand-bottom sections, increase through material of larger size, and reach a maximum in coarse gravel and rubble and in mud suitable for burrowing forms. This is true in the comparative sense, but absolute production figures may vary widely between two streams sampled in what seems, by present knowledge, closely similar situations. I should like to submit some of my own hitherto-unpublished data by way of illustration. Table 1 lists the total macroinvertebrate fauna found in a representative square-foot sample taken from the North Branch of the Au Sable River, generally considered Michigan's most productive trout stream. Table 2 lists contents of a similar sample from the West Branch of the Sturgeon River, a stream notably low in production. The North Branch sample yielded 1, 374 mayfly

Table 1. STATION 1185, NORTH BRANCH OF AU SABLE RIVER, CRAWFORD CO., MICHIGAN, FEBRUARY 25, 1935

Name	Number	Volume, cm^3
Tubifex	7	Trace
Glossiphonia	7	0.025
Dugesia	7	0.025
Physa	1	0.025
Ancylus	1	Trace
Pisidium	110	0.125
Sphaerium	4	0.050
Orconectes propinquus	1	3.000
Gammarus fasciatus	18	0.400
Hyalella azteca	19	0.025
Heptagenia hebe	17	0.025
Stenonema fuscum	43	
Paraleptophlebia mollis	26	
Baetis cingulatus	11	
Ephemerella invaria, subvaria	1,277	2.400
Allocapnia torontoensis	8	0.050
Isoperla signata	7	0.050
Isoperla slossonae	23	Trace
Stenelmis	80	0.100
Phryganea cinerea	1	Trace
Rhyacophila manistee	1	Trace
Hydropsyche sparna, slossonae	120	0.750
Pycnopsyche lepida	11	1.850
Helicopsyche borealis	8	0.025
Lepidostoma togatum	23	0.100
Antocha saxicola	58	1.000
Tendipedidae	302	0.300
Heleidae	37	Trace
Atherix variegata	1	0.025
Hydrodarina	1	Trace
Totals, 1 square foot	2,230	10.250

nymphs; of these, 1,277 were two closely related species of *Ephemerella*, *invaria* and *subvaria*, which are difficult to distinguish except in late instars. The sample shown in Table 2 contains much the same association of species, but the number of individuals and volume of nymphs are negligible by comparison.

Table 4 presents rather detailed chemical analyses of water from (1) the Au Sable system, (2) the Sturgeon system, and (3) the Ontonogon system. Both (1) and (2) are typical hard-water streams that flow through glacial drift topography in the northern Lower Peninsula; (3) is a soft-water stream characteristic of the Pre-Cambrian formations of the western Upper Peninsula and is between (1) and (2) in trout production.

Table 2. STATION 1160, WEST BRANCH OF STURGEON RIVER, CHEBOYGAN CO., MICHIGAN, JANUARY 21, 1935

Name	Number	Volume, cm^3
Physa	1	Trace
Pisidium	1	Trace
Emphemerella invaria, subvaria	43	0.075
Paraleptophlebia mollis	1	
Baetis cingulatus	17	
Baetis vagans	5	
Heptagenia hebe	1	
Rhithrogena jejuna	14	
Capnia vernalis	5	0.025
Isoperla dicala	15	
Hydroperla olivacea	1	
Stenelmis	15	0.025
Glossosoma americana	290	0.075
Rhyacophila manistee	3	
Micrasema rusticum	3	
Oxyethira sp.	3	
Anthocha saxicola	7	0.025
Simulium venustum	3	
Tendipedidae	12	
Atherix variegata	2	
Totals, 1 square foot	442	0.225

Table 3, showing the mayfly yields of samples taken from the North Branch in February, April, and May, is included here chiefly to show that changes in the mayfly fauna are what might be expected from normal seasonal succession.

It is difficult to account for the wide disparity in bottom-fauna production between (1) and (2) on the basis of water chemistry. The physical characteristics of the two streams are superficially quite similar, and the bottom samples were taken in gravel of closely comparable size. About the only visible difference is that the gravel in the West Branch of the Sturgeon is smooth and polished, while that in

Table 3. COMPARATIVE OCCURRENCE OF EPHEMEROPTERA NYMPHS IN NORTH BRANCH OF AU SABLE RIVER, MICHIGAN, LATE WINTER TO SPRING (1935)

	February 25		April 25		May 19	
	Number	Volume	Number	Volume	Number	Volume
Ephemerella invaria and subvaria	1,277	2.400	711	2.100	157	0.900
Ephemerella lata	0	0	68	0.050	87	0.150
Paraleptophlebia mollis	26	Trace	54	0.050	18	0.025
Baetis cingulatus	11	Trace	23	0.050	0	0
Heptagenia hebe	17	Trace	43	0.025	0	0
Stenonema fuscum	43	0.025	85	0.200	1	Trace
Stenonema canadense	0	0	27	0.025	61	0.025

Table 4. WATER ANALYSES FOR THREE MICHIGAN STREAMS OF VARYING PRODUCTIVITY SAMPLED FROM AUGUST 19 - SEPTEMBER 2, 1958, BETWEEN 1:00 AND 4:00 p.m. [a]

Stream	Temp, °F,		Oxygen,	Carbon dioxide,	Alkalinity,		
	Water	Air	ppm	ppm	Phenolphthalein	Methyl orange	pH
Au Sable, Crawford Co.	54	68	9.0	0.0	6.0	155	8.2
Sturgeon, Cheboygan Co.	57	68	10.9	0.0	5.0	181	8.4
Ontonogon, Gogebic Co.	69	80	12.0	0.0	3.0	83	7.4

Stream	Conductivity, μ mho, 18°C	Sodium, ppm	Potassium, ppm	Calcium, ppm	Magnesium, ppm	Iron, ppb	Aluminum, ppm	Silica, ppm
Au Sable, Crawford Co.	256	0.9	0.7	52.2	7.9	5	tr	5.0
Sturgeon, Cheboygan Co.	305	1.5	0.6	71.3	12.0	172	tr	6.0
Ontonogon, Gogebic Co.	151	1.2	1.1	26.3	5.3	172	0.04	8.1

Stream	Nitrogen, ppm	Phosphorus (total soluble), ppb		Halogen (total), ppm	Chloride, ppm	Sulfate, ppm
Au Sable, Crawford Co.	0.64	13.9	1.7	3.6	3.55	9.2
Sturgeon, Cheboygan Co.	1.26	20.3	1.9	1.2	0.89	12.6
Ontonogon, Gogebic Co.	1.04	33.1	2.0	---	---	7.8

[a] From Urshel and Hopper, 1961.

the North Branch of the Au Sable is thickly encrusted with a porous, easily crumbled, calcareous deposit. This deposit supports occasional tufts of *Fissidens* on its rough, irregular surface, and both the surface and the porous interior offer much more harborage for organisms than does the smooth surface of the bare gravel of the Sturgeon. Further, the porosities of the Au Sable deposits are lined with algal growth. To use a rather crude comparison, a section of the deposit resembles a slice of Roquefort cheese.

We have been unable to devise a method of computing the total surface available to organisms, per square foot of bottom, in the North Branch. But far more baffling is our inability to explain the presence of the calcareous deposit there and its absence from the West Branch of the Sturgeon.

Table 5 represents an attempt to show the ecological distribution of mayfly nymphs in Michigan trout streams. The categories used are rather broad but are practical and helpful for the field collector and fish management worker.

My purpose in outlining this problem is to emphasize the inadequacy of our knowledge of the environmental requirements of mayflies. There is a clear need for intensive work on microhabitats and for evaluation of the effects of different types of pollution on the habitat and the individual species themselves. Present knowledge of mayfly taxonomy and life cycles, while far from complete, provides an adequate foundation for such intensive study.

Table 5. ECOLOGICAL DISTRIBUTION OF MAYFLY NYMPHS IN MICHIGAN TROUT STREAMS

Sand and gravel (burrowing nymphs)

Ephemera simulans

Mud (burrowing nymphs)

Hexagenia limbata
Hexagenia atrocaudata (beaver ponds)
Hexagenia recurvata (cold streams)
Hexagenia rigida (warm streams)

Gravel and rubble

Ephemerella cornuta (Pre-Cambrian bedrock streams)
Ephemerella dorothea
Ephemerella excrucians
Ephemerella invaria
Ephemerella lata
Ephemerella rotunda
Ephemerella subvaria

Table 5. ECOLOGICAL DISTRIBUTION OF MAYFLY NYMPHS IN MICHIGAN TROUT STREAMS (CONTINUED)

Paraleptophlebia adoptiva
Paraleptophlebia debilis
Paraleptophlebia mollis
Paraleptophlebia praepedita (warm streams)
Baetis brunneicolor
Baetis cingulatus
Baetis intercalaris (also plant beds)
Baetis vagans
Pseudocloeon anoka
Epeorus vitreus (fast water only)
Rhithrogena jejuna
Rhithrogena impersonata
Rhithrogena pellucida
Rhithrogena sanguinea

Underside of stones

Ephemerella walkeri (more often in leaf drift)
Stenonema canadense (also in leaf drift in fast water)
Stenonema fuscum
Stenonema frontale (also in leaf drift)
Stenonema integrum (fast water only)
Stenonema ithaca
Stenonema luteum (fast water only)
Stenonema nepotellum (fast water only)
Stenonema pulchellum (more often on submerged logs)
Stenonema vicarium (also in leaf drift)
Heptagenia hebe (oftener in leaf drift)

Submerged plant beds

Ephemerella needhami (also leaf drift and submerged logs)
Callibaetis ferrugineus (quiet water only)
Baetis hiemalis (quiet water only)
Baetis intercalaris (oftener in gravel riffles)
Baetis pygmaeus

Leaf drift and detritus, nonburrowing nymphs

Ephemerella deficiens
Ephemerella needhami (also in submerged plant beds)
Ephemerella walkeri (also under stones)
Stenonema canadense (also under stones)
Stenonema frontale (also under stones)
Stenonema pulchellum (prefers submerged logs)
Stenonema tripunctatum
Stenonema vicarium (also under stones)
Heptagenia hebe (also under stones)
Heptagenia pulla

Detritus, little or no current

Tricorythodes spp. (cold streams)
Brachycercus lacustris (warm streams)
Caenis forcipata (spring-holes)
Caenis jocosa
Caenis simulans
Ephemerella deficiens
Ephemerella lutulenta
Ephemerella needhami (also leaf drift and plant beds)
Ephemerella simplex
Ephemerella temporalis
Baetisca laurentina (cold streams)
Baetisca obesa (warm streams)
Leptophlebia cupida
Leptophlebia nebulosa
Stenonema heterotarsale

Quiet water, free-swimming nymphs

Siphlonurus alternatus
Siphlonurus quebecensis
Siphlonurus rapidus
Centroptilum album (warm streams)
Cloeon sp.
Metretopus borealis (Pre-Cambrian bedrock streams)

Moderate to fast current, free-swimming nymphs

Isonychia bicolor
Isonychia harperi
Isonychia sadleri
Siphloplecton basale

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ENVIRONMENTAL REQUIREMENTS OF TRICHOPTERA

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INTRODUCTION

I should like, in this talk, to present some of the tolerances to chemical factors and limitations of the caddisfly fauna of our streams and rivers. The purely physical habitat preferences are well reported in the literature (Ross, 1949; Betten, 1934; Lloyd, 1921) and need not be repeated here; Hynes (1960) has summarized the data on caddisfly tolerances and habitat to that date. It was felt to be more pertinent to present these previously unreported data that have been gleaned from over 10 years of stream survey reports.

Before going into the chemistry, we might examine the importance of the caddisfly larvae as an element of stream fauna and the relative abundance of the commonly found families. An examination of the species lists from our surveys revealed that the caddisfly larvae formed 8 to 13 percent of the fauna of any river studied. The average was about 10 percent.

Table 1 gives the relative abundance of the common caddisfly families from the aspects of both frequency of occurrence and population. The caddisfly fauna of 10 rivers, ranging from Center Creek, Missouri, to the St. Lawrence at Brockville, were tabulated. The last column is a subjective estimate of the relative abundance, based on my experience. The table shows that the first four families dominate the caddisfly fauna both from frequency of occurrence and population. The fifth family, the Philopotamidae, represented by the genus *Chimarra,* though having few species per river, is usually abundant where present - more so than the Psychomyiidae or Limnephilidae.

CHEMISTRY

Tables 2 through 15 were made by taking the chemical data from about 100 stations on 25 rivers in the United States and Canada and recording the occurrence of each of the genera involved at each of these stations. These data were coded and fed into a computer that gave the number of times each genus occurred at a certain range for each chemical. For example, in Table 2 *Chimarra* appeared 3 times at stations with an alkalinity of 10 ppm or less, 7 times at alkalinity between 11 and 20 ppm, 5 times between 21 and 30 ppm, etc. While the rivers studied do represent a good cross-section of the rivers in the United States and Canada, there is a bias introduced into these tables because certain rivers have been studied more frequently than others, and this tends to elevate certain columns artifically: Most of the tables represent 600 or more records.

Alkalinity - M.O.: Table 2 shows that alkalinity per se is not a particularly significant factor in the appearance of caddisfly larvae. Most of the genera can tolerate a range of from about 20 to 100 ppm. The peaks in distribution at concentrations of 30 and 100 ppm are artificial. They are caused by the more frequent collection from certain rivers at those ranges.

Table 1. RELATIVE ABUNDANCE OF COMMON CADDISFLY FAMILIES OF 10 RIVERS

Caddisfly families	Number of rivers	Species per rivers	Estimated relative abundance, percentage
Hydropsychidae	9	3.9	80
Psychomyiidae	8	2.4	3
Limnephilidae	8	2.1	2
Leptoceridae	7	5.0	10
Philopotamidae	5	1.6	4
Hydroptilidae	7	1.7	0.1
Brachycentridae	3	1.0	0.2
Glossosomatidae	2	1.0	0.1
Helicopsychidae	1	1.0	0.2
Molannidae	1	1.0	0.1
Lepidostomatidae	1	1.0	0.1
Phryganeidae	2	1.5	0.2
			100

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Table 2. FREQUENCY OF OCCURRENCE OF CADDISFLY GENERA AT DIFFERENT LEVELS OF METHYL ORANGE ALKALINITY (669 RECORDS)

Caddisfly genera	Methyl orange alkalinity, ppm								
	10	20	30	40	50	100	150	200	550
Chimarra	3	7	5				1	1	
Neureclipsis	1	7	8	5		18	6	4	2
Phylocentropus	5	2	2			2	4		
Polycentropus		2	2	2		10	2	1	1
Psychomyiid sp.			13	4	1	13		2	1
Cheumatopsyche	3	12	8	4	2	24	8	3	1
Hydropsyche	2	14	18	11	4	32	9	11	6
Macronemum	3	14	21	5	1	21	6		
Hydroptila	1	5	3			12	1	2	2
Athripsodes		6	8	3		12		1	
Leptocella		11	16	2	2	19	7	3	3
Mystacides						4	4		
Oecetis	2	7	8	1	1	22	7	5	3
Triaenodes	2	3	6	1		6	6	1	1
Pycnopsyche	4	5		1		15	4	1	3
Ptilostomus					1	3			
Molanna	1	2				4	1		
Helichopsyche		1				9		2	2
Brachycentrus	5	4	1			6	1		
Lepidostoma	3	1							
Totals	35	103	119	39	12	232	67	37	25

Cl^-: Table 3 shows that 8 of the 20 genera can tolerate a chloride concentration of over 2,500 ppm. This is equivalent to a salinity concentration of 4,500 ppm. This is within the mesohaline category of Redeke (1922). The net makers appear to be slightly more common at these high salinities. The genus labelled Psychomyiid sp. is close to Psychomyiid genus A Ross and is relatively common at stations with a high salinity.

Table 3. FREQUENCY OF OCCURRENCE OF CATTISFLY GENERA AT DIFFERENT CHLORIDE LEVELS (671 RECORDS)

Caddisfly genera	Chloride concentration, ppm								
	1	3	5	10	50	100	500	2,500	10,000
Chimarra	1	5	2	7	1				1
Neureclipsis	1	9	13	5	16	5	1		1
Phylocentropus	2	4	1	2	6				
Polycentropus		2	1	2	14			1	
Psychomyiid sp.		6		16	6	2	1	2	1
Cheumatopsyche	1	8	13	15	25	2			2
Hydropsyche	2	17	17	24	32	11	1		3
Macronemum	4	18	16	15	16	1	1		
Hydroptila		8	4	8	6	1			
Athripsodes		11	8	5	5	1			
Leptocella	1	10	11	17	18	3	1		2
Mystacides					8				
Oecetis		5	8	18	22	2			
Triaenodes		3	4	8	9				2
Pycnopsyche	1	7	5	5	15				1
Ptilostomus		1		2	1				
Molanna		2		2	4				
Helichopsyche			4	1	9				
Brachycentrus	2	6	1	2	6				
Lepidostoma		4							
Totals	15	126	108	154	219	28	5	3	13

CO_2: In table 4 we see that eight genera can tolerate CO_2 concentrations over 40 ppm. The gap from 21 to 40 ppm is artificial because no stations collected had CO_2 concentrations in that range. Most of the genera found about 40 ppm are case makers.

DO: Only 6 of the 20 genera occurred at a DO level of less than 3 ppm (Table 5). The gap at 3 is artificial. *Cheumatopsyche* and *Hydropsyche* have been recorded as tolerant of organic pollution and their presence at low DO concentrations is not surprising. The presence of *Molanna* and *Helichopsyche*, is, however, unusual. These genera are usually found in clear, cold streams with a sand or gravel bottom.

Table 4. FREQUENCY OF OCCURRENCE OF CADDISFLY LARVAE AT DIFFERENT CARBON DIOXIDE LEVELS (371 RECORDS)

Caddisfly genera	Carbon dioxide concentrations, ppm						
	1	5	10	20	30	40	50
Chimarra	1	5	9	1			1
Neureclipsis	1	10	18				
Phylocentropus		1	5	1			1
Polycentropus		2	4				
Psychomyiid sp.		9	12				
Cheumatopsyche	1	11	19	1			
Hydropsyche	1	28	36	1			
Macronemum		15	29	1			
Hydroptila		5	8				
Athripsodes		7	11				
Leptocella	1	10	24	1			
Mystacides							
Oecetis		8	18	1			1
Triaenodes	1	4	7	1			1
Pycnopsyche		4	8	1			1
Ptilostomus		1					
Molanna		1		2			1
Helichopsyche		2	3				
Brachycentrus		2	6	1			1
Lepidostoma			3				1
Totals	6	125	220	12	0	0	8

Table 5. FREQUENCY OF OCCURRENCE OF CADDISFLY GENERA AT DIFFERENT DISSOLVED OXYGEN LEVELS (601 RECORDS)

Caddisfly genera	Dissolved oxygen concentrations, ppm						
	1	3	5	7	9	11	15
Chimarra				4	9	4	
Neureclipsis			1	12	22	9	
Phylocentropus				1	12	2	
Polycentropus			1	2	4	13	
Psychomyiid sp.				16	13		
Cheumatopsyche	1		2	12	24	19	1
Hydropsyche	1		3	29	36	27	1
Macronemum			1	15	36	12	
Hydroptila				6	10	4	2
Athripsodes	1			3	16	6	
Leptocella	1		2	20	20	13	
Mystacides					4	4	
Oecetis			2	16	19	12	
Triaenodes			1	7	11	3	
Pycnopsyche				2	20	7	1
Ptilostomus				1		3	
Molanna	1				4	3	
Helichopsyche	1			1	5	3	
Brachycentrus				1	9	7	
Lepidostoma					2	2	
Totals	6	0	13	148	276	153	5

Fe: Like alkalinity, iron (Table 6) is of practically no significance in the distribution of caddisfly larvae. Most of the genera occur over the entire range found in the streams and rivers studied. Even some of the less common genera were found over a wide range of iron concentrations (cf. *Lepidostoma)*.

Total Hardness: Only 4 of the genera listed (Table 7) were found at hardness values above 2,500 ppm. The gap at from 1,001 to 2,500 ppm is unfortunate, but we can presume that the genera found above 2,500 ppm would also be found there. Only one of four genera above 2,500 ppm was a case maker.

Table 6. FREQUENCY OF OCCURRENCE OF CADDISFLY GENERA AT DIFFERENT LEVELS OF IRON (422 RECORDS)

Caddisfly genera	Iron concentration, ppm							
	0.01	0.03	0.05	0.07	0.1	0.4	0.7	1.0
Chimarra	2	2	3		1	5	2	1
Neureclipsis	7	5	6	2	1	7	2	
Phylocentropus		2	2	1	3	1	1	1
Polycentropus	2	4	1	1	1	2		1
Psychomyiid sp.	2	1	6	1	1	3	2	4
Cheumatopsyche	6	9	5	4	2	9	3	
Hydropsyche	9	14	14	4	3	15	3	1
Macronemum	8	7	10	2	7	8	1	
Hydroptila	4	6	6	1	1	4	1	
Athripsodes	4	1	5	1	3	3	1	1
Leptocella	6	8	3	2	2	15	1	1
Mystacides	1	2						
Oecetis	5	7	5	1	2	7	3	1
Triaenodes	2	1	2	1		5	2	1
Pycnopsyche	2	7	3	1	1	5		
Ptilostomus		3				1		
Molanna		3	1	1			2	
Helicopsyche		5	1	1	1			
Brachycentrus		3	3	2	2	1	2	
Lepidostoma			1	1	1	1		
Totals	60	95	77	27	32	92	26	12

Table 7. FREQUENCY OF OCCURRENCE OF CADDISFLY GENERA AT DIFFERENT LEVELS OF TOTAL HARDNESS (667 RECORDS)

Caddisfly genera	Total hardness, ppm									
	5	10	50	100	150	200	500	1,000	2,500	5,000
Chimarra	1	3	10		1			1		1
Neureclipsis		2	18	7	9	7	7			
Phylocentropus		5	4		2	4				
Polycentropus			5	1	4	7	2	1		
Psychomyiid sp.			14	10	4		3	3		
Cheumatopsyche		2	24	10	12	10	5	1		2
Hydropsyche	1	6	36	17	13	12	16	3		3
Macronemum	1	6	36	8	10	8	2			
Hydroptila			9	9	2	3	1	3		
Athripsodes		2	15	4	8		1			
Leptocella	1	2	24	12	7	8	7			
Mystacides				1	2	5				
Oecetis		1	16	12	7	11	6	2		
Triaenodes		1	9	4	4	4	2			2
Pycnopsyche	1	3	5	4	12	4	2	2		
Ptilostomus			1	2	1					
Molanna	1	1	1		4	1				
Helichopsyche			1	4	4	1	1	3		
Brachycentrus		5	5		3	4				
Lepidostoma		2	2							
Totals	6	41	235	105	109	89	55	19	0	8

NH_3-N: The caddisfly larvae (Table 8) appear to be well distributed over the range from <0.001 ppm to 1.0 ppm. • Above this they drop off and only four genera are found at levels above 10.0 ppm. The presence of *Brachycentrus*, a cool-clear-water form, at NH_3 concentrations above 10.0 is surprising.

NO_3-N: Unlike NH_3, the distribution records in Table 9 are largely clustered in the (0.014 ppm to 0.7 ppm) middle range of concentrations. Here there appears to be both upper and lower limits to the optional concentrations of NO_3. There are only scattered records below 0.014 ppm and above 0.7 ppm.

Table 8. FREQUENCY OF OCCURRENCE OF CADDISFLY GENERA AT DIFFERENT AMMONIA NITROGEN LEVELS (665 RECORDS)

Caddisfly genera	Ammonia nitrogen concentrations, ppm						
	0.001	0.009	0.03	0.05	1.0	10.0	20.0
Chimarra	2	4	5	2	2	2	
Neureclipsis	9	13	9	5	14	1	
Phylocentropus	5	3	2	1	3	1	
Polycentropus	4	4	2		10		
Psychomyiid sp.	4		1	1	26	2	
Cheumatopsyche	10	16	16	3	17	2	
Hydropsyche	14	15	24	7	42	3	1
Macronemum	12	14	12	5	26	1	1
Hydroptila		6	3	1	15	1	
Athripsodes	1	12	7	3	6	1	
Leptocella	8	15	16	2	18	3	1
Mystacides	4		3	1			
Oecetis	9	11	11	2	21	2	
Triaenodes	5	10	4		4	3	
Pycnopsyche	8	8	6	3	5	2	
Ptilostomus		3			1		
Molanna	1	2	2		2	1	
Helichopsyche		5	3		5		
Brachycentrus	5	2	4	1	4		1
Lepidostoma		2	1	1			
Totals	101	145	131	38	221	25	4

Table 9. FREQUENCY OF OCCURRENCE OF CADDISFLY GENERA AT DIFFERENT LEVELS OF NITRATE NITROGEN (665 RECORDS)

Caddisfly genera	Nitrate nitrogen concentrations, ppm								
	0.001	0.007	0.013	0.03	0.07	0.2	0.7	1.5	3.0
Chimarra	1				2	8	6		
Neureclipsis				1	12	21	17		
Phylocentropus			1	1	4	3	6		
Polycentropus				3	4	8	3		2
Psychomyiid sp.				2	14	11	2	5	
Cheumatopsyche		1		2	13	23	23	1	1
Hydropsyche	1			3	20	43	29	3	2
Macronemum	1	1			12	40	17		
Hydroptila				3	1	9	8	5	
Athripsodes				1	7	15	7		
Leptocella	1			4	8	35	15		
Mystacides				1	2		4	1	
Oecetis				4	8	26	18		
Triaenodes				2		12	12		
Pycnopsyche	1				7	12	12		
Ptilostomus							1	1	2
Molanna	1			1	3	1	2		
Helichopsyche				1	4	4	4		
Brachycentrus		1			4	9	3		
Lepidostoma					2	1	1		
Totals	6	3	1	29	127	286	190	16	7

pH: Most of the genera in Table 10 are recorded at pH values between 6.0 and 9.0. None of the rivers collected has a pH value more than 9 or in the 3.0 to 5.5 range. We can presume that since six genera are represented at pH < 3.0, some of these would carry through to 5.5 if collections were made in suitable streams or rivers.

PO_4: The distribution records at different phosphate levels (Table 11) are spread from 0.002 to 0.5 ppm. Below 0.002, only four genera of net makers are found. Above 0.5, especially at 5.0 ppm, the case-maker genera are more common. Only *Hydropsyche* carries through the entire range.

Table 10. FREQUENCY OF OCCURRENCE OF CADDISFLY GENERA AT DIFFERENT HYDROGEN IRON LEVELS (666 RECORDS)

Caddisfly genera	pH values								
	3.0	4.5	5.0	5.5	6.0	6.5	7.5	8.0	9.0
Chimarra					1	2	10	3	
Neureclipsis						1	20	19	10
Phylocentropus	1				1	5	3		6
Polycentropus							6	4	10
Psychomyiid sp.							20	13	1
Cheumatopsyche	1					4	22	20	19
Hydropsyche						2	48	36	20
Macronemum	1					4	39	16	10
Hydroptila						1	10	11	5
Athripsodes	1						11	8	5
Leptocella							31	18	13
Mystacides							1		7
Oecetis	1				1	2	16	22	14
Triaenodes					1	2	7	12	4
Pycnopsyche					1	3	4	17	8
Ptilostomus							2		2
Molanna					1	1		1	4
Helichopsyche							1	8	5
Brachycentrus	1				1	6	1	1	7
Lepidostoma					1	2	1		
Totals	6	0	0	0	8	35	258	209	150

Table 11. FREQUENCY OF OCCURRENCE OF CADDIFSLY GENERA AT DIFFERENT PHOSPHATE LEVELS (646 RECORDS)

Caddisfly genera	Phosphate concentrations, ppm								
	0.001	0.005	0.01	0.05	0.1	0.5	1.0	5.0	10.0
Chimarra			1	9	2	5			
Neureclipsis	2	4	9	14	18	3			
Phylocentropus		2		6	6				
Polycentropus		2	3	6	5	4			
Psychomyiid sp.		18	1	2	9	4			
Cheumatopsyche	1	8	8	18	15	12	1		
Hydropsyche	3	15	13	27	21	19	2	2	1
Macronemum	3	12	8	21	20	2		1	
Hydroptila		2	2	6	10	5		1	
Athripsodes		2	6	13	6	2			
Leptocella		7	5	19	17	12		1	
Mystacides				3	4	1			
Oecetis		7	7	17	14	10		1	
Triaenodes		4	4	6	7	4		1	
Pycnopsyche		2	4	7	11	4			
Ptilostomus				2		1		1	
Molanna				4	1	3			
Helichopsyche		2	5	4		2			
Brachycentrus		1		6	6	2		1	
Lepidostoma				2	1				
Totals	9	88	76	192	173	95	3	9	1

Temperature: Temperature, at least at the gradients found in our work (Table 12), does not seem to be a limiting factor. Most of the genera are well distributed from < 12° to 35° C.

SO_4: A preponderance of genera is found at sulfate levels in the 2 to 60 ppm range. Above this there are scattered records up to 480 ppm, with only Psychomyiid sp. being above 480 ppm. Below 10 ppm the records, as can be seen, are also rather sparse (Table 13).

Table 12. FREQUENCY OF OCCURRENCE OF CADDISFLY GENERA AT DIFFERENT TEMPERATURE LEVELS (644 RECORDS)

Caddisfly genera	Temperature, °C							
	12	17	20	23	28	30	35	40
Chimarra	1			3	13			
Neureclipsis	2	1	5	5	27	2	7	
Phylocentropus		1	2	5	7			
Polycentropus		3	2	3	6		2	
Psychomyiid sp.		1	5	2	17	7		
Cheumatopsyche	1	5	8	15	28	2	5	
Hydropsyche	7	4	5	19	50	9	11	
Macronemum	5	3	7	10	36	5	5	
Hydroptila	1		2	3	12	4		
Athripsodes	2	1	5	8	9	1	4	
Leptocella	1	2	5	10	27	10	7	
Mystacides				3	4			
Oecetis		1	6	5	25	9	7	
Triaenodes			2	2	14	3	4	
Pycnopsyche	3	3	1	9	13			
Ptilostomus		1	1	2				
Molanna	1			4	2			
Helichopsyche		1		8			4	
Brachycentrus		1	3	6	7			
Lepidostoma		1	1	1	1			
Totals	24	29	60	125	298	52	56	

Table 13. FREQUENCY OF OCCURRENCE OF CADDISFLY GENERA AT DIFFERENT SULFATE LEVELS (619 RECORDS)

Caddisfly genera	Sulfate concentrations, ppm									
	1	10	30	60	90	120	240	480	960	1,500
Chimarra	1	10	4			1		1		
Neureclipsis		18	16	11	3	1				
Phylocentropus		7	6		1					
Polycentropus		3	8	4	2		3			
Psychomyiid sp.	1	6	11	4			1	2	1	
Cheumatopsyche		17	22	14	4	1	3	1		
Hydropsyche	1	29	28	22	4	2	5	5		
Macronemum	1	32	8	16	3					
Hydroptila		7	15	1				4		
Athripsodes		15	5	7	1					
Leptocella	1	16	23	11	1	2	2			
Mystacides			8							
Oecetis		13	21	11	3		2	2		
Triaenodes		6	7	5	3	1	2			
Pycnopsyche	1	8	8	11	2		2	2		
Ptilostomus		1	2	1						
Molanna	1	3	4							
Helichopsyche		1	5	4			1	3		
Brachycentrus		9	2	5						
Lepidostoma		4								
Totals	7	205	203	127	27	8	21	20	1	

Turbidity: The caddisfly larvae seem to be able to tolerate turbidity levels up to 100 ppm (Table 14). Above this they drop off slightly at 500 ppm (16 genera present). Between 501 and 1,000 ppm the records are fewer; only seven genera are present. There does not appear to be any difference between the case makers and the net makers in response. One would normally expect that the net makers, which are dependent on suspended material for their food supply, would be more sensitive to high turbidities since the silt load would tend to clog their food-gathering nets.

BOD: The caddisfly larvae (Table 15) are well distributed at levels up to 5.0 ppm. The paucity of

Table 14. FREQUENCY OF OCCURRENCE OF CADDISFLY GENERA AT DIFFERENT TURBIDITY LEVELS (667 RECORDS)

Caddisfly genera	Turbidity levels, ppm					
	10	25	50	100	500	1,000
Chimarra	5	4	5	2	1	
Neureclipsis	4	11	11	16	8	
Phylocentropus	5	1	7	1	1	
Polycentropus	5	5	3	6	1	
Psychomyiid sp.	7	8	7	11	1	
Cheumatopsyche	12	13	19	13	7	1
Hydropsyche	18	23	20	29	13	2
Macronemum	9	14	18	17	10	2
Hydroptila	3	10	4	7	3	
Athripsodes	4	5	7	7	5	2
Leptocella	7	12	18	14	11	1
Mystacides	3		5			
Oecetis	7	13	14	15	6	1
Triaenodes	3	7	11	3	2	
Pycnopsyche	7	7	7	8	4	1
Ptilostomus		2	2			
Molanna	5	2		1		
Helichopsyche	6	6	1	1		
Brachycentrus	6	2	3	5	1	
Lepidostoma	1	1	1		1	
Totals	117	146	163	156	75	10

Table 15. FREQUENCY OF OCCURRENCE OF CADDISFLY GENERA AT DIFFERENT BIOCHEMICAL OXYGEN DEMAND LEVELS (581 RECORDS)

Caddisfly genera	BOD levels, ppm						
	0.1	0.5	1.0	5.0	10.0	20.0	40.0
Chimarra		2	8	6			1
Neureclipsis	4	10	15	15	1		
Phylocentropus		2	6	6			
Polycentropus			6	7	1	4	
Psychomyiid sp.		8	5	5			
Cheumatopsyche	4	4	21	21	3	4	
Hydropsyche	6	14	30	30	5	4	1
Macronemum	4	8	26	16	2	4	1
Hydroptila	1	4	4	11	1		
Athripsodes		4	15	8	2		
Leptocella	4	5	19	20	1	4	1
Mystacides			2	6			
Oecetis	3	4	16	20	1	4	
Triaenodes		3	9	11	1		
Pycnopsyche		7	13	10	2		
Ptilostomus				3			
Molanna		2	3	2			
Helichopsyche		2	9	3			
Brachycentrus		2	5	4		4	
Lepidostoma		2	1	1			
Totals	28	83	213	205	20	28	4

records at the low end is ascribable to a lack of streams with BOD so low. Above 5.0 ppm the records are more sparse, with only four genera above 20.0 ppm. *Cheumatopsyche* has been recorded in the literature as tolerant of organic load. As can be seen in Table 15, four genera, three of them net makers, were found at higher BOD levels than *Cheumatopsyche*.

Cu and Zn: We have only one set of field data, from the North Anna River in Virginia, in which tests were conducted for soluble zinc and copper. In this situation we found *Chimarra* and *Cheumatopsyche* at soluble zinc concentrations of 0.02 ppm and soluble copper concentrations of 0.056 ppm. *Macronemum* and *Brachycentrus* were found at soluble zinc concentrations of <0.004 and soluble copper concentrations of <0.020 ppm. Psychomyiid sp. was present at soluble zinc levels of 0.006 ppm and copper levels of 0.000, dropped out at zinc 0.02 ppm and copper 0.056, and reappeared at zinc 0.014 ppm and copper 0.036 ppm.

TOXICITY TESTS

This is a field in which a great deal of work needs to be done. We need to know more of the effects of the factors discussed under controlled conditions and of the effects of combinations of these factors. As far as insects are concerned, toxicity tests must be interpreted with caution for the following reasons: Difficulty in keeping specimens alive; general lack of strong movement (compared with fish) and the consequent lack of mixing in the solution; creation of microclimates around each specimen; the plating out effect of herby metals, which increases local concentrations; determination of the instar being tested; and the difficulty in rearing through several generations to show long-term sublethal effects.

Table 16 shows the results of a series of toxicity tests conducted by Dr. Benoit at the Academy of Natural Sciences. The specimens were kept in the lids of 2-pound butter dishes that were placed in a temperature-controlled room. The water used was a modification of Chu 14. The *Hydropsyche* were *H. betteni* and *Hydropsyche (bifida* group) spp. I have included the data on mayflies (*Stenonema rubrum* McD) for the purpose of comparison. The differences between the two genera in tolerating the chemicals listed are quite striking, the caddisfly larvae being far more tolerant than *Stenonema*. The 9,000 ppm NaCl is equivalent to a Cl concentration of 5,400 ppm. Converted to salinity, this is 9,750 ppm salinity - just below the lower limit of the polyhaline category of Redeke (1922).

SUMMARY

We can say that caddisfly larvae are tolerant of a wide range of most of the chemical factors reported. *Cheumatopsyche* and *Hydropsyche* have been reported tolerant of a wide range of chemical conditions, particularly pollution. This is borne out by the data presented here. Both were found at DO values as low as 1.0 ppm. *Cheumatopsyche* was found in water containing over 10 ppm BOD and *Hydropsyche* at over 20 ppm BOD. In addition, both were tolerant of high turbidity, SO_4 concentrations over 240 ppm, total hardness over 2,500 ppm, and Cl concentrations over 2,500 ppm. Other of the genera also proved to be quite tolerant. *Chimarra* was found in water containing over 2,500 ppm of Cl, over 40 ppm of CO_2, over 2,500 ppm hardness, and over 20 ppm BOD. *Macronemum* and *Leptocella* were found over a wide range of most of the chemical factors reported. In general there were few differences in response between the net and web builders versus the case-making genera. For CO_2, more case makers were found at concentrations over 40 ppm; the net builders were more common at high hardness values and low PO_4 concentrations. At higher phosphate concentrations (over 1.0 ppm), the case makers were more abundant, and at high BOD levels the net builders were more common. The records at these extremes are so sparse that one must be careful not to exaggerate the significance of these differences.

Table 16. TOLERANCE OF *HYDROPSYCHE* AND *STENONEMA* TO VARIOUS CHEMICALS. 100 PERCENT SURVIVAL IN ALL CONTROLS

Chemical	Temp, °F	Dil Water	48-hr TL_m ppm	
			Hydropsyche	*Stenonema*
NaCl	68-72	Soft	9,000	2,500
$K_2Cr_2O_7$ as Cr	68-72	Soft	280	3.5
KCN (as CN)	68-72	Soft	2.0	0.5
Phenol	68-72	Soft	30	14.5

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A SURVEY OF ENVIRONMENTAL REQUIREMENTS FOR THE MIDGE

(Diptera: Tendipedidae)

*By LaVerne L. Curry**

INTRODUCTION

A discussion of the environmental requirements of many organisms will include habitats ranging from stock-watering tanks to vernal ponds. Likewise, the fauna found in the variety of habitats is diverse. Probably one of the most rewarding experiences of the biologist is to glean general knowledge from the samples after the organisms have been identified.

There is a pressing need today for information-physical, chemical, biological-regarding all phases of aquatic life. A deluge of wastes is entering waters, and the effects upon the biomass are little known. Many examples can be cited, but a case in point is that of the Clear Lake gnat in California (Kipling, 1950; Lindquist and Roth, 1950; Walker, 1951). In the treatment of this lake, the tendipedids were virtually eliminated during the first season, but appeared again, resulting in additional treatment programs. Cost of the original project was $42,000, and the cost for each retreatment program, even higher.

Today biologists are investigating the plausibility of applying natural controls in conjunction with artificial methods. For example, comparisons are being made with the use of carp. *(Cyprinus carpio* Linne, goldfish *(Carassius auratus* Linne) , catfish *(Ictalurus punctatus* Rafinesque) , and exotic fish, such as *Tilapia mossambica* Peters, as predators on tendipedids (Bay and Anderson, 1960). Results of these tests are being compared with the effectiveness of larvicides.

It is apparent that the biologist must be aware of environmental limitations when applying biological controls. In many cases, however, our present knowledge is still incomplete regarding the problem species itself. The purpose of this paper is to present physical, chemical, and biological data regarding the fresh-water tendipedid fauna. Some of the information is derived from the literature, since the data obtained from field studies in Michigan fell within the arbitrary limits established in this paper.

HISTORY

At present, little is known of the ecological requirements of fresh water tendipedids. Considerable effort has been expended to obtain data from occasional studies. Early investigations were general, and data pertaining to tendipedids were incidental to the overall project, whether it was in fisheries, lake systems, or pollution (Hankinson, 1908; Malloch, 1915; Baker, 1918; Richardson, 1928). Later, Brundin (1949) investigated the relationship of tendipedids with respect to lake typology, and Thienemann (1954) recorded considerable information about tendipedids throughout the world.

In recent years, the problem of tendipedid identification has been given much-needed attention. A revision of the family has been made by Townes (1945) and Johannsen, et al. (1952). Information is now available for the biologist of pollution on the Ethiopian and Australian fauna (Freeman, 1955, 1956, 1957, 1958, 1961). With the continued interest in public health problems by the World Health Organization, this information will find an appropriate use.

Problems in nomenclature have posed a problem for water pollution biologists. Since investigations are carried on in different biogeographical regions and two diametrically opposed taxonomic systems are used, the problem is more acute. Additional keys have been published for many of the immature stages and for adult tendipedids since the work of Johannsen (1937a, 1937b). These include information on the biology of the tendipedid species in the Philadelphia area (Roback, 1957) and Michigan (Curry, 1958).

The nomenclature employed in this paper is based upon that of Townes (1945) and Johannsen, et al. (1952). For convenience in using European literature, a synonymy checklist is derived for the Nearctic forms of the genus *Tendipes* when known (Table 1). This list is based, in part, upon the nomenclature employed by Edwards (1929).

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Table 1. SYNONYMY CHECKLIST OF EARLY EUROPEAN AND AMERICAN AUTHORS

Allochironomus Kieffer = *Tanytarsus (Stictochironomus)* Kieffer
annularis Macquart = *riparius* (Meigen, *Tandipes (T)*
anomalus Kieffer = *riparius* (Meigen), *Tendipes (T)*
anonymous Williston, *Chironomus* = *attenuatus* (Walker), *Tendipes (T)*
anthracinus ?, Tendipes = *anthracinus* (Zetterstedt), *Tendipes (T)*
anthracinus Zetterstedt, *Chironomus* = *anthracinus* (Zetterstedt), *Tendipes (T)*
atritibia Malloch, *Chironomus (C)* = *atritibia* (Malloch), *Tendipes (T)*
attenuatus Walker, *Chironomus* = *attenuatus* (Walker), *Tendipes (T)*
bathophilus Kieffer, *Chironomus* = *anthracinus* (Zetterstedt, *Tendipes (T)*
brevitibialis (Goetghebuer), *Chironomus* = *lobiger* (Kieffer), *Tendipes (L)*
brevitibialis (Kieffer), *Chironomus* = *lobiger* (Kieffer), *Tendipes (L)*
brevitibialis (Zetterstedt), *Chironomus* = *nervosus* (Staeger), *Tendipes (L)*
Brunieria Kieffer = *Tanytarsus (Stictochironomus)* Kieffer
brunneipennis Johannsen, *Chironomus* = *brunneipennis* (Johannsen), *Tendipes (E)*
californicus (Johannsen), *Chironomus* = *californicus* (Johannsen), *Tendipes (L)*
Camptochironomus Kieffer = *Tendipes (Tendipes)* Meigen
camptolabis Kieffer, *Cladopelma* = *Harnischia (Cladopelma)* Kieffer
cayugae Johannsen, *Chironomus* = *attenuatus* (Walker), *Tendipes (T)*
Chironomus Meigen = *Tendipes* Meigen
cingulatus Meigen, *Chironomus* = *stigmaterus* (Say), *Tendipes (T)*
Cladopelma Kieffer = *Harnischia (cladopelma)*
Cladotendipes Lenz
Clinochironomus Kieffer = *Tendipes* Meigen
conformis Malloch, *Chironomus* = *pilicornis* (Fabricius), *Tendipes (T)*
crassicaudatus Beyer ?, *Tendipes* = *crassicaudatus* Malloch, *Tendipes (T)*
cristatus ? Fabricius, *Chironomus* = *attenuatus* (Walker) *Tendipes (T)*
cristatus Fabricius ?, *Chironomus* = *attenuatus* (Walker) *Tendipes (T)*
curtibarba Kieffer = *riparius* (Meigen), *Tendipes (T)*
curtiforceps Kieffer = *riparius* (Meigen), *Tendipes (T)*
decorus Johannsen, *Chironomus* = *attenuatus* (Walker) *Tendipes (T)*
decorus Johannsen, *Chironomus (C)* = *attenuatus* (Walker), *Tendipes (T)*
decorus Johannsen, *Tendipes* = *attenuatus* (Walker) *Tendipes (T)*
decumbens Malloch, *Chironomus (C)* = *decumbens* (Malloch, *Tendipes (T)*
dichromocerus Kieffer ? = *riparius* (Meigen), *Tendipes (T)*
distans Kieffer ? = *riparius* (Meigen), *Tendipes (T)*
distinguendus Kieffer, *Chironomus* = *attenuatus* (Walker), *Tendipes (T)*
dolens Walker ?, *Chironomus (C)* = *pilicornis* (Fabricius), *Tendipes (T)*
Dolichopelma Kieffer = *Lauterborniella* Bause
dorsalis (? not Meigen), *Chironomus* = *riparius* (Meigen), *Tendipes (T)*
dorsalis Meigen, *Chironomus* = *dorsalis* (Meigen), *Tendipes (E)*
dorsalis Meigen, *Chironomus* = *riparius* (Meigen), *Tendipes (T)*
dorsalis Meigen, *Tendipes* = *dorsalis* (Meigen) *Tendipes (E)*
dux Johannsen, *Chironomus* = *dux* (Johannsen), *Tendipes (K)*
dux Johannsen, *Cladopelma* = *dux* (Johannsen, *Tendipes (K)*
falciformis Kieffer, *Chironomus* = *nervosus* (Staeger), *Tendipes (L)*
fasciventris Malloch, *Chironomus* = *staegeri* (Lundbeck) *Tendipes (T)*
fasciventris Malloch?, *Chironomus (C)* = *staegeri* (Lundbeck), *Tendipes (T)*
ferrugineo-vittatus Zetterstedt, *Chironomus* = *ferrugineo-vittatus* (Zetterstedt), *Tendipes (T)*
ferrugineo-vittatus Johannsen, *Chironomus* = *plumosus* (Linnaeus), *Tendipes (T)*
fulvipilus Rempel, *Chironomus* = *fulvipilus* (Rempel), *Tendipes ? (T)*
fumidus (Johannsen), *Chironomus* = *fumidus* (Johannsen), *Tendipes (L)*
fumidus Johannsen, *Chironomus (L)* = *neomodestus* (Malloch), *Tendipes (L)*
futilis (Walker), *Chironomus* = *nervosus* (Staeger), *Tendipes (L)*
glaucurus Wiedemann, *Chironomus* = *stigmaterus* (Say), *Tendipes (T)*
goetghebueri Kieffer, *Chironomus* = *nervosus* (Staeger), *Tendipes (L)*
Goetghebueria Kieffer = *Tenytarsus* Wulp
gregarius Kieffer, *Chironomus* = *riparius* (Meigen), *Tendipes (T)*
grisescens Goetghebuer, *Chironomus* = *riparius* (Meigen), *Tendipes (T)*
helochares Kieffer, *Chironomus* = *riparius* (Meigen), *Tendipes (T)*
hyperboreus Staeger, *Chironomus* = *anthracinus* (Zetterstedt), *Tendipes (T)*
hyperboreus Staeger, *Chironomus* = *hyperboreus* (Staeger), *Tendipes (T)*
hyperboreus Staeger, *Chironomus (C)* = *anthracinus* (Zetterstedt), *Tendipes (T)*
hyperboreus Staeger, *Chironomus (C)* = *hyperboreus* (Staeger), *Tendipes (T)*
hyperboreus Staeger, *Tendipes (T)* = *hyperboreus* (Staeger), *Tendipes (T)*
hyperboreus variety *meridionalis* Johannsen, *Chironomus* = *anthracinus* (Zetterstedt), *Tendipes (T)*

Table 1. SYNONYMY CHECKLIST OF EARLY EUROPEAN AND AMERICAN AUTHORS (CONTINUED)

hyperboreus variety Staeger, *Chironomus* = *staegeri* (Lundbeck), *Tendipes (T)*
imperator Walley, *Chironomus* = *plumosus* (Linnaeus), *Tendipes (T)*
incognitus (Malloch), *Chironomus* = *fumidus* (Johannsen), *Tendipes (L)*
indistunctus Malloch, *Chironomus* = *nervosus* (Staeger), *Tendipes (L)*
indivisus Kieffer = *riparius* (Meigen), *Tendipes (T)*
interruptus Kieffer = *riparius* (Meigen), *Tendipes (T)*
iththybrota Kieffer = *riparius* (Meigen), *Tendipes (T)*
Limnotendipes Lenz = *Tendipes (Limnochironomus)* Kieffer
lobiger Kieffer *Chironomus* = *lobiger* (Kieffer), *Tendipes (L)*
lobiger variety *miriforceps* (Kieffer), *Tendipes (L)* - *lobiger* (Kieffer), *Tendipes (L)*
longipes Staeger, *Chironomus* = *dorsalis* (Meigen), *Tendipes (E)*
lucifer Johannsen, *Chironomus* = *nervosus* (Staeger), *Tendipes (L)*
lucifer Johannsen, *Chironomus (C) (Limnochironomus* group) = *nervosus* (Staeger), *Tendipes (L)*
maturus Johannsen, *Chironomus* = *attenuatus* (Walker), *Tendipes (T)*
meridionalis Johannsen?, *Chironomus* = *anthracinus* (Zetterstedt), *Tendipes (T)*
militaris Johannsen, *Chironomus* = *riparius* (Meigen), *Tendipes (T)*
miriforcips (Kieffer), *Chironomus* = *lobiger* (Kieffer), *Tendipes (T)*
miriforcips (Goetghebuer), *Chironomus* = *lobiger* (Kieffer), *Tendipes (T)*
modestus Say, *Chironomus (Limnochironomus)* = *modestus* (Say), *Tendipes (L)*
modestus (Say), *Cladopelma* = *modestus* (Say), *Tendipes (L)*
modestus variety a, *Chironomus* = *nervosus* (Staeger), *Tendipes (L)*
moerens Walker?, *Chironomus (C)* = *pilicornis* (Fabricius), *Tendipes (T)*
neomodestus Malloch, *Chironomus* = *neomodestus* (Malloch), *Tendipes (T)*
nervosus Staeger, *Chironomus* = *nervosus* (Staeger), *Tendipes (T)*
nervosus Staeger, *Chironomus (L)* = *nervosus* (Staeger), *Tendipes (L)*
niger Meigen, *Chironomus (C)* = *pilicornis* (Fabricius), *Tendipes (T)*
niveipennis Fabricius, *Chironomus* = *pilicornis* (Fabricius), *Tendipes (T)*
obscuratus Malloch, *Chironomus* = *dux* (Johannsen), *Tendipes (K)*
paganus Meigen, *Chironomus* = *paganus* (Meigen), *Tendipes (E)*
paganus Meigen, *Tendipes (Phytotendipes)* = *paganus* (Meigen), *Tendipes (E)*
Phytochironomus Kieffer = *Glyptotendipes (Glyptodentipes)* Kieffer
Phytotendipes Goetghebuer = *Glyptotendipes (Phytotendipes)* Goetghebuer
pilicornis ?, *Chironomus (C)* = *pilicornis* (Fabricius), *Tendipes (T)*
pilicornis Fabricius, *Chironomus* = *pilicornis* (Fabricius), *Tendipes (T)*
plumosa Linnaeus, *Tipula* = *plumosus* (Linnaeus), *Tendipes (T)*
plumosus Linnaeus, *Chironomus* = *plumosus* (Linnaeus), *Tendipes (T)*
plumosus Linnaeus, *Tendipes* = *plumosus* (Linnaeus), *Tendipes (T)*
plumosus variety *ferrugineo-vittatus* Johannsen = *ferrugineo-vittatus* (Zetterstedt), *Tendipes (T)*
plumosus variety *ferrugineo-vittatus* Johannsen, *Chironomus* = *plumosus* (Linnaeus), *Tendipes (T)*
polaris Kirby?, *Chironomus* = *hyperboreus* (Staeger), *Tendipes (T)*
polaris Kirby, *Tendipes (T)* = *hyperboreus* (Staeger), *Tendipes (T)*
Prochironomus Kieffer = *Tanytarsus* Wulp
Protanytarsus Kieffer = *Tanytarsus* (genus)
restrictus Kieffer? = *riparius* (Meigen), *Tendipes (T)*
rhyparobius Kieffer = *riparius* (Meigen), *Tendipes (T)*
riparius Kieffer?, *Tendipes* = *riparius* (Meigen), *Tendipes (T)*
riparius Kruseman nec. Meigen = *riparius* (Meigen), *Tendipes (T)*
riparius Meigen, *Chironomus* = *riparius* (Meigen), *Tendipes (T)*
riparius Meigen, *Chironomus* = *staegeri* (Lundbeck), *Tendipes (T)*
saxonicus Kieffer = *riparius* (Meigen), *Tendipes (T)*
serus Malloch, *Chironomus* = *riparius* (Meigen), *Tendipes (T)*
serus? Johannsen, *Chironomus (C)* = *riparius* (Meigen), *Tendipes (T)*
similis Johannsen, *Chironomus* = *attenuatus* (Walker), *Tendipes (T)*
staegeri Lundbeck, *Chironomus* = *staegeri* (Lundbeck), *Tendipes (T)*
staegeri Lundbeck?, *Chironomus (C)* = *staegeri* (Lundbeck), *Tendipes (T)*
staegeri? Lundbeck, *Chironomus* = *atritibia* (Malloch), *Tendipes (T)*
Stictotendipes Lenz = *Tendipes (Tendipes)* Meigen
stigmaterus Say, *Chironomus* = *stigmaterus* (Say), *Tendipes (T)*
stigmaterus? Say, *Chironomus* = *stigmaterus* (Say), *Tendipes (T)*
stricticornis Kieffer = *riparius* (Meigen), *Tendipes (T)*
subacutus Kieffer = *riparius* (Meigen), *Tendipes (T)*
subproductus Kieffer = *riparius* (Meigen), *Tendipes (T)*
subrectus Kieffer = *riparius* (Meigen) *Tendipes (T)*
subriparius Kieffer = *riparius* (Meigen), *Tendipes (T)*
Syntendipes Lenz = *Tendipes (Tendipes)* Meigen

Table 1. SYNONYMY CHECKLIST OF EARLY EUROPEAN AND AMERICAN AUTHORS (CONTINUED)

tenellus Zetterstedt, *Chironomus* = *neomodestus* (Malloch), *Tendipes (L)*
tentans Fabricius, *Chironomus* = *plumosus* (Linnaeus), *Tendipes (T)*
tentans Fabricius, *Chironomus* = *tentans* (Fabricius), *Tendipes (T)*
tentans Kruseman?, *Tendipes (Camptochironomus)* = *tentans* (Fabricius), *Tendipes (T)*
tentans variety *pallidivittatus* Malloch, *Chironomus* = *tentans* (Fabricius), *Tendipes (T)*
thummi Kieffer, *Chironomus* = *riparius* (Meigen), *Tendipes (T)*
thummi Kieffer, *Tendipes* = *riparius* (Meigen), *Tendipes (T)*
tristis Wiedemann, *Chironomus (C)* = *pilicornis* (Fabricius), *Tendipes (T)*
tritomus Kieffer, *Chironomus* = *nervosus* (Staeger), *Tendipes (L)*
tuxis Curran, *Chironomus* = *tuxis* (Curran), *Tendipes (T)*
tuxis Curran?, *Chironomus (C)* = *tuxis* (Curran), *Tendipes (T)*
?*tuxis* Curran, *Tendipes (T)* = *tuxis* (Curran), *Tendipes (T)*
utahensis Malloch, *Chironomus* = *utahensis* (Malloch), *Tendipes (T)*
viridicollis Wulp *Chironomus* = *atroviridis* Townes, *Tendipes (C)*
viridicollis Wulp, ?*Chironomus* = *atroviridis* Townes, *Tendipes (C)*
viridicollis ? Wulp, *Chironomus* = *riparius* (Meigen), *Tendipes (T)*
(near) *viridicollis* Wulp, *Chironomus* = *atroviridis* Townes, *Tendipes (C)*
"*viridicollis*" Wulp, *Chironomus (E)* = *atroviridis* Townes, *Tendipes (C)*
viridipes Macquart = *riparius* (Meigen), *Tendipes (T)*

The following names have no synonymy.
aethiops Townes, *Tendipes (L)*
atrella Townes, *Tendipes (T)*
biseta Townes, *Tendipes (T)*
botarus Townes, *Tendipes (L)*
carvs Townes, *Tendipes (T)*
Chaetolabis Townes
chelonia Townes, *Tendipes (E)*
Einfeldia Kieffer
Kiefferulus Goetghebuer
leucoscelis, Townes, *Tendipes (L)*
milleri Townes, *Tendipes (L)*
ochreatus Townes, *Tendipes (C)*
pungens Townes, *Tendipes (T)*
tendipediformis Goetghebuer, *Tendipes (K)*
tuberculatus Townes, *Tendipes (T)*

ENVIRONMENTAL REQUIREMENTS

Evidence has been introduced describing types of environmental selection by the fresh-water tendipedids. These data have been applied in developing a lake "typology" (Brundin, 1949; Thienemann, 1954) that has found some application in the United States. Richardson (1928) proposed a classification, based on tolerance to pollution, of tendipedids found in the middle Illinois River. Later work by Paine and Gaufin (1956) questioned the use of tendipedid larvae as indicator organisms because of their adaptability to environmental conditions.

This adaptability is illustrated by their morphology and physiology. The larval adaptations were used by Leathers (1922) to classify the members of the family into six groups (1) burrowers, (2) attached tubes, (3) tubes of mud, (4) leaf-eating, (5) alga-eating, and (6) free-living larvae. With respect to their physiology, tendipeded larvae have been collected in habitats ranging from hot springs (Brues, 1928) to salt-water rock pools (Palmen, 1956; Lindeberg, 1958). In addition, field and laboratory work has been conducted on selective larvicides, based on tendipedid biology, in Wisconsin and New York (Dicke, 1957; Bay, 1960). It is not unusual then to find the larval forms existing in a great variety of environmental conditions.

The field work conducted in the Central Michigan area did not involve grossly polluted streams. In an attempt to determine the maximum-minimum requirements of tendipedids, the literature was used extensively. Unfortunately, only a small portion of it was useful because the forms were not classified as to genus or species. The maximum-minimum levels of the environmental conditions found in this study are as follows:

Temperature. In general, the temperature requirements of the tendipedids are within the limits established by the Aquatic Life Advisory Committee (1956) in that "aquatic organisms in the temperate zone are adapted to seasonal fluctuations of temperature between 0° and 32°C (32° to about 90°F)" (Table 2). One species, *Pelopia*, is able to live under an extreme temperature of 39.59°C (103°F). The upper limit, in general, for the majority of species in the subfamilies Pelopiinae, Diamesinae, Hydrobaeninae and Tendipedinae is between 30 to 33°C (86 to 93.2°F). Larvae of *Cricotopus trifasciatus* (Panzer), *C. bicinctus* (Meigen), as well as representatives of *Pentaneura* and *Cryptochironomus* are reported in waters having temperatures of 34°C (93.2°F). Immature forms of *Pentaneura monilis* (Linnaeus) have been found in waters having temperatures of 35°C (95°F). In

Table 2. RANGES OF ENVIRONMENTAL FACTORS FOR FRESH-WATER TENDIPEDID SPECIES

Species	Temperature, °C min	max	pH, min max	DO, ml/per liter, min	CO_2, ppm max	Authors
Pelopiinae						
Pentaneura carnea (Fabricius)	0.0	32.8	6.0 to 9.1	1.0		Paine (1956), Roback (1957)
Pentaneura illinoesis (Malloch)	0.0	32.8	7.2 to 9.1	1.0		Paine (1956)
Pentaneura melanops (Wiedemann)	0.0	32.8	7.2 to 9.1	1.0		Paine (1956)
Pentaneura monilis (Linné)	4.4	35.0	6.0 to 8.0		46.0	Brues (1922), Roback (1957)
Pentaneura vitellina (Kieffer)	0.0	32.8	7.2 to 9.1	1.0		Paine (1956)
Penaneura sp.	4.4	34.0	5.2 to 8.5			Jones (1948), Wurtz (1960)
Anatopynia dyari (Coquillett)	0.0	32.8	6.0 to 9.1	1.0		Paine (1956), Roback (1957)
Anatopynia sp.						Jones (1949)
Pelopia punctipennis (Meigen)	0.0	32.8	7.2 to 9.1	1.0		Paine (1956)
Pelopia stellata (Coquillett)	0.0	32.8	7.2 to 9.1	1.0		Guyer (1956), Paine (1956)
Pelopia vilipennis (Kieffer)	4.4	30.0	6.8 to 8.5	0.5	61.0	
Pelopia sp.		39.5	8.1			Brues (1928)
Procladius bellus Loew	4.4	26.7	7.0 to 8.5	0.0	46.0	Guyer (1956)
Procladius choreus Meigen	4.4	26.7	7.0 to 8.5	0.0	46.0	
Procladius culciformis (Linné)	0.0	32.8	6.0 to 9.1	1.0		Paine (1956), Roback (1957)
Procladius sp.	0.0	30.0	6.8 to 9.1	1.0	61.0	Dicke (1957), Jones (1949), Lindeberg (1958), Paine (1956), Teter (1960)
Clinotanypus caliginosus (Johannsen)	0.0	32.8	7.2 to 9.1	1.0		Paine (1956)
Coelotanypus concinnus (Coquillett)	0.0	32.8	7.2 to 9.1	1.0		Paine (1956)
Diamesinae						
Prodiamesa olivacea (Meigen)			4.4 to 7.8			Jones (1948)
Syndiamesa						
Diamesa nivoriunda Fitch	0.0	32.8	7.2 to 9.1	1.0		Paine (1956)
Hydrobaeninae						
Corynoneura scutellata (Winnertz)	0.0	32.8	7.2 to 9.1	1.0		Paine (1956)
Corynoneura sp.	0.0	32.8	4.4 to 9.1	1.0		Jones (1948), Paine (1956), Lindeberg (1958)
Cricotopus absurdus (Johannsen)	0.0	32.8	7.2 to 9.1	1.0		Paine (1956)
Cricotopus bicinctus (Meigen)	0.0	34.0	6.0 to 9.1	1.0		Baker (1918), Roback (1957), Paine (1956, Wurtz (1960)
Cricotopus exilis (Johannsen)	0.0	32.8	7.2 to 9.1	1.0		Paine (1956)
Cricotopus politus (Coquillett)	0.0	32.8	7.2 to 9.1	1.0		Paine (1956)
Cricotopus tricinctus (Meigen)	0.0	32.8	7.2 to 9.1	1.0		Paine (1956)
Cricotopus trifasciatus (Panzer)	0.0	34.0	6.0 to 9.1	1.0		Paine (1956), Roback (1957), Wurtz (1960)
Cricotopus sp.		38.8	9.6			Brues (1928), Jones (1948)(1949)
Hydrobaenus sp.	0.0	34.0	6.8 to 9.1		61.0	Brues (1928), Paine (1956), Jones (1948) (1949)
Tendipedinae						
Calopsectra dives (Johannsen)	4.4	26.7	7.0 to 8.5		46.0	Teter (1960)
Calopsectra johannseni (Bause)	0.0	32.8	7.2 to 9.1	1.0		Paine (1956)
Calopsectra neaflavella (Malloch)	0.0	32.0	7.2 to 9.1	1.0		Paine (1956)
Calopsectra nigripilus (Johannsen)	0.0	32.8	7.2 to 9.1	1.0		Paine (1956)
Calopsectra sp.	0.0	30.0	6.8 to 9.1		61.0	Dicke (1957), Paine (1956), Teter (1960)
Pseudochironomus richardsoni (Malloch)	0.0	32.8	7.2 to 9.1			Paine (1956)
Pseudochironomus sp.	4.4	30.0	6.8 to 8.5		61.0	
Lauterborniella gracilenta			4.4 to 7.8			Jones (1948)
Lauterborniella varipennis (Coquillett)	4.4	26.7	6.0 to 8.5		46.0	
Microtendipes pedelius (DeGeer)	0.0	32.8	6.0 to 9.1	1.0	46.0	Paine (1956), Roback (1957)
Microtendipes sp.	7.8					Jones (1949)
Paratendipes albimanus (Meigen)	0.0	32.8	5.0 to 9.1	1.0	46.0	Lindeberg (1958), Paine (1956), Roback (1957)

Table 2. RANGES OF ENVIRONMENTAL FACTORS FOR FRESH-WATER TENDIPEDID SPECIES (CONTINUED)

Species	Temperature, °C,		pH,		DO, ml/liter	CO_2, ppm	Authors
	min	max	min	max	min	max	
Tendipedinae (Continued)							
Paratendipes sp.		38.7		8.1			Brues (1928)
Polypedilum fallax (Johannsen)	0.0	32.8	6.0 to	9.1	1.0		Paine (1956, Roback (1957)
Polypedilum halterale (Coquillett)	4.4	30.0	5.0 to	8.5		61.0	Roback (1957)
Polypedilum illinoense (Malloch)	0.0	32.8	4.0 to	9.1	1.0		Paine (1956), Roback (1957), Wurtz (1960)
Polypedilum nubeculosum (Meigen)	4.4	26.7	7.0 to	8.5		46.0	Palmen (1956)
Polypedilum simulans Townes	4.4	40.0	6.8 to	8.5		61.0	Guyer (1956)
Polypedilum sp.	4.4	30.0	6.8 to	8.5		61.0	Teter (1960)
Tanytarsus devinctus (Say)	4.4	26.7	7.0 to	8.5		46.0	Paine (1956), Roback (1957)
Tanytarsus jucundus (Walker)	4.4	26.7	4.0 to	8.5		46.0	Roback (1957)
Tanytarsus nigricans (Johannsen)	0.0	32.8	6.0 to	9.0	1.0	61.0	Paine (1956), Roback (1957)
Tanytarsus varius Townes	0.0	32.8	7.2 to	9.1	1.0		Paine (1956)
Tanytarsus sp.							Jones (1949)
Xenochironomus scopula Townes	0.0	32.8	7.0 to	9.1	1.0		Paine (1956)
Cryptochironomus blarina Townes	4.4	26.7	7.0 to	8.5		46.0	Curry (1958)
Cryptochironomus digitatus (Malloch)	3.0	30.0		8.8			Curry (1958), Guyer (1956), Lindeberg (1958), Tebo (1955)
Cryptochironomus fulvus (Johannsen)	0.0	32.8	4.0 to	9.1	1.0	46.0	Curry (1958), Paine (1956), Roback (1957)
Tendipes anthracinus (Zetterstedt)							Lindeberg (1958)
Tendipes atroviridis Townes	4.4	30.0	6.8 to	8.5		61.0	Teter (1960), Roback (1957)
Tendipes attenuatus (Walker)	0.0	32.8	4.0 to	9.1		61.0	Dicke (1957), Paine (1956), Lindeberg (1958), Palmen (1956)
Tendipes dux (Johannsen)	0.0	32.0	6.8 to	9.1	1.0	61.0	Paine (1956)
Tendipes fumidus (Johannsen)	4.4	26.7	5.0 to	9.0		46.0	Roback (1957)
Tendipes milleri Townes	4.4	26.7	7.0 to	8.5		46.0	Roback (1957)
Tendipes modestus (Say)	4.4	26.7	5.0 to	9.0		46.0	Roback (1957)
Tendipes neomodestus (Malloch)	0.0	32.8	7.0 to	9.1	1.0	46.0	Paine (1956), Roback (1957)
Tendipes nervosus (Staeger)	4.4	30.0	6.9 to	8.5		61.0	Guyer (1956), Palmen (1956)
Tendipes ochreatus Townes	4.4	30.0	6.8 to	8.5		61.0	
Tendipes paganus (Meigen)	4.4	27.0	7.0 to	8.5		46.0	
Tendipes plumosus (Linne)	3.0	30.0	6.8 to	8.8		61.0	Dicke (1957)
Tendipes riparius (Meigen)	0.0	32.8	5.0 to	9.1		61.0	Paine (1956), Roback (1957), Wurtz (1960)
Tendipes staegeri (Lundbeck)	4.4	30.0	6.0 to	8.5	0.0	61.0	Roback (1957)
Tendipes tendipediformis (Goetghebuer)	4.4	30.0	6.8 to	8.5	0.0	61.0	
Tendipes tentans (Fabricius)	4.4	35.0	6.8 to	8.5	0.0	61.0	Brues (1928), (Lindeberg (1958)
Tendipes tuxis (Curran)	4.4	27.0	7.0 to	8.5		46.0	
Tendipes sp.							Jones (1949)
Glyptotendipes barbipes (Staeger)							Lindeberg (1958)
Glyptotendipes lobiferus (Say)	0.0	32.8	7.0 to	9.1	1.0	46.0	Paine (1956)
Glyptotendipes paripes (Edwards)	4.4	27.0	5.0 to	8.5		46.0	Guyer (1956), Warnhoff (1957)
Glyptotendipes sp.	0.0	32.8	7.0 to	9.1	1.0	46.0	Dicke (1957), Paine (1956), Wurtz (1960)
Harnischia tenuicaudata (Malloch)	4.4	30.0	6.0 to	8.5		61.0	

addition, larvae of the genera of *Pelopia*, *Cricotopus*, and *Parantendipes* have been found in waters having temperatures of 38° to 39.5°C (100° to 103°F) (Brues, 1928; Jones, 1948, 1949; Paine and Gaufin, 1956; Roback 1957; and Wurtz, 1960). The minimal environmental temperature reported for larval habitats is at or near 0°C.

Hydrogen ion concentration. Many stream forms are able to endure a pH range of 6.0 to 8.0 or higher (Table 2). Representatives of the genera *Pentaneura*, *Anatopynia*, *Pelopia*, *Procladius*, *Clinotanypus*, *Diamesa*, *Corymoneura*, *Cricotopus*, and *Hydrobaenus* have been collected in habitats having a pH 9.1 (Jones, 1948; Paine and Gaufin, 1956; Lindeberg, 1958; Wurtz, 1960). Several members of the *Calopsectrini* are also reported to inhabit waters having a pH 9.1. Many members of the tribe *Tendipedini* have been found in waters having hydrogen ion concentrations of this level. Representative genera for these forms are

Pseudochironomus, Microtendipes, Paratendipes, Polypedilum, Tanytarsus, Tendipes, and *Glyptotendipes.*

The species *Prodiamesa olivacea* (Meigen), a stream form, has been found inhabiting water having a pH 4.4 (Jones, 1948). The species *Lauterborniella gracilenta, Polypedilum illinoense* (Malloch), *Tanytarsus jucundus* (Walker), *Cryptochilenta, Polypedilum illinoense* (Malloch), *Tanytarsus jucundus* (Walker), *Cryptochironomus fulvus* (Johannsen), and *Tendipes attenuatus* (Walker) [=*Tendipes decorus* (Johannsen)] are reported from waters having a pH 4.0.

Dissolved oxygen. Frequently this is listed as a limiting factor in a given environment. A consideration of this ecological requirement should also take into account factors such as temperature, pollution, and metabolic activity of the organism.

It appears that very few tendipedids are able to withstand prolonged anaerobic conditions imposed by some lakes and streams (Table 2). Additional information is required through the use of bioassays (Aquatic Advisory Committee, 1956). Species of *Pentaneura, Pelopia, Clinotanypus, Coelotanypus, Corynoneura,* and *Cricotopus* can, however, endure a dissolved-oxygen content of 1.1 cm^3 per liter. In Michigan, *Pelopia vilipennis* Kieffer was found inhabiting a hydrosol where the oxygen of the superimposed water was 0.5 cm^3 per liter.

Many species of the Tendipedinae are also found to inhabit waters with low dissolved-oxygen content. These forms are found in all the major genera. Only forms such as *Tendipes tentans* (Fabricius); T. *staegeri* (Lundbeck); T. *riparius* (Meigen); T. *plumosus* (L); and *T. attenuatus* are, however, reported inhabiting the anaerobic region of lakes and streams. Studies on populations of two tendipedid forms in Michigan lakes indicate further that the mortality rate is high for the species found there. This mortality may run as much as 50 percent. The greatest mortality apparently is in the first or second instars of larval development.

Carbon dioxide. Very few species of the subfamilies Pelopiinae, Diamesinae, or Hydrobaeninae are reported inhabiting waters where high concentrations of carbon dioxide are found (Table 2). Larvae of *Pentaneura monilis, P. vilipennis, Pelopia bellus* Loew, P. *choreus* Meigen, and a species of *Hydrobaenus* have been found in waters having 46 to 61 ppm CO_2.

Many species of the subfamily Tendipedinae appear to inhabit hydrosols with superimposed waters having a high concentration of carbon dioxide. The most conspicuous forms are those of the genus *Tendipes*. This genus appears to have the majority of forms adapted in living under these environmental conditions.

Ionic concentration. Very little has been recorded for a comparative study of tolerance to ionic concentrations such as Ca^{++}, $PO_4^{\equiv}$, $SO_4^{=}$, and trace elements (Table 3). The comparative physiological resistance to chloride ions illustrates, however, a physiological adaptability attained by a few species to salt water. Palmén (1956) and Lindeberg (1958) have reported the *Procladius* sp., *Corynoneura* sp, *Microtendipes pedellus, Paratendipes albimanus* (Meigen), *Polypedilum halterale* (Coquillett), *P. nubeculosum* (Meigen), *Tendipes anthracinus* (Zetterstedt), *T. attenuatus, T. nervosus* (Staeger), and *T. tentans* inhabit brackish water. For a better understanding of the adaptability of the organisms, bioassay tests should be run on representative larvae of the various genera.

Table 3. RANGES OF ENVIRONMENTAL FACTORS FOR FRESH-WATER TENDIPEDID SPECIES

Species	$PO_4^{\equiv}$, ppm, total max	Ca^{++}, ppm, max	Mg^{++}, ppm, max	Fe^{+++}, ppm, max	Cl^-, ppm, max	$SO_4^=$, ppm, max	NO_3^-, ppm, total max	$CO_3^=$, ppm, max	BOD, max	Authors
Pelopiinae										
Pentaneura carnae (Fabricius)	26.2						43.0	310		Paine (1956), Roback (1957)
Pentaneura illinoensis (Malloch)	26.2						43.0	310	82.0	Paine (1956)
Pentaneura melanops (Weidemann)	26.2						43.0	310	82.0	Paine (1956)
Pentaneura monilis (Linne)										Brues (1922), Roback (1957)
Pentaneura vitellina (Kieffer)	26.2						43.0	310	82.0	Paine (1956)
Pentaneura sp.		25.8	5.0	tr [a]	8.8	9.0			3.04	Jones (1948), Wurtz (1960)
Anatopynia dyari (Coquillett)	26.2						43.0	310	82.0	Paine (1956), Roback (1957)
Anatopynia sp.		22.0	4.0	0.0	8.5	1.0				Jones (1949)
Pelopia punctipennis (Meigen)	26.2						43.0	310	82.0	Paine (1956)
Pelopia stellata (Coquillett)	26.2						43.0	310	82.0	Guyer (1956), Paine (1956)
Pelopia vilipennis (Kieffer)										

Table 3. RANGES OF ENVIRONMENTAL FACTORS FOR FRESH-WATER TENDIPEDID SPECIES (CONTINUED)

Species	$PO_4^{\equiv}$, ppm, total max	Ca^{++}, ppm, max	Mg^{++}, ppm, max	Fe^{+++}, ppm, max	Cl^-, ppm, max	$SO_4^=$, ppm, max	NO_3^-, ppm, total max	$CO_3^=$, ppm, max	BOD, max	Authors
Pelopiinae (continued)										
Pelopia sp.										Brues (1928)
Procladius bellus Loew										Guyer (1956)
Procladius choreus Meigen										
Procladius culciformis (Linne)	26.2						43.0	310	82.0	Paine (1956),Roback (1957)
Procladius sp.	26.2	22.0	4.0	0.0	6.0	1.0	43.0	310	82.0	Dicke (1957),Jones (1949), Lindeberg (1958), Paine (1956), Teter (1960)
Clinotanypus caliginosus (Johannsen)	26.2						43.0	310	82.0	Paine (1956)
Coelotanypus concinnus (Coquillett)	26.2						43.0	310	82.0	Paine (1956)
Diamesinae										
Prodiamesa olivacea (Meigen)		23.0	4.5	0.0	12.0	tr				Jones (1948)
Syndiamesa sp.		22.0	4.0	0.0	8.5	1.0				
Diamesa nivoriunda Fitch	26.2						43.0	310	82.0	Paine (1956)
Hydrobaininae										
Corynoneura scutellata (Winnertz)	26.2						43.0	310	82.0	Paine (1956)
Corynoneura sp.		23.0	4.5	0.0	†	tr	43.0	310	82.0	Jones (1948), Paine (1956), Lindeberg (1958)
Cricotopus absurdus (Johannsen)	26.2						43.0	310	82.0	Paine (1956)
Cricotopus bicinctus (Meigen)	26.2						43.0	310	82.0	Baker (1918), Roback (1957), Paine (1956), Wurtz (1960)
Cricotopus exilis (Johannsen)	26.2						43.0	310	82.0	Paine (1956)
Cricotopus politus (Coquillett)	26.2						43.0	310	82.0	Paine (1956)
Cricotopus tricinctus (Meigen)	26.2						43.0	310	82.0	Paine (1956)
Cricotopus trifasciatus (Panzer)	26.2						43.0	310	82.0	Paine (1956), Roback (1957), Wurtz (1960)
Cricotopus sp.		25.8	5.0	0.0	12.0	tr			3.04	Brues (1928), Jones (1948) (1949)
Hydrobaenus sp.		25.8	5.0	0.0	12.0	1.0	43.0	310	82.0	Brues (1928), Paine (1956), Jones (1948) (1949)
Tendipedinae										
Calopsectra dives (Johannsen)										Teter (1960)
Calopsectra johannseni (Bause)	26.2						43.0	310	82.0	Paine (1956)
Calopsectra neoflavella (Malloch)	26.2						43.0	310	82.0	Paine (1956)
Calopsectra nigripilus (Johannsen)	26.2						43.0	310	82.0	Paine (1956)
Calopsectra sp.	26.2						43.0	310	82.0	Dicke (1957), Paine (1956), Teter (1960)
Pseudochironomus richardsoni (Malloch)	26.2						43.0	310	82.0	Paine (1956)
Pseudochironomus sp.										

Table 3. RANGES OF ENVIRONMENTAL FACTORS FOR FRESH-WATER TENDIPEDID SPECIES (CONTINUED)

Species	$PO_4^{\equiv}$, ppm, total max	Ca^{++}, ppm, max	Mg^{++}, ppm, max	Fe^{+++}, ppm, max	Cl^-, ppm, max	$SO_4^=$, ppm, max	NO_3^-, ppm, total max	$CO_3^=$, ppm, max	BOD, max	Authors
Tendipedinae (continued)										
Lauterborniella gracilenta										Jones (1948)
Lauterborniella varipennis (Coquillett)										
Microtendipes pedellus (DeGeer)	26.2				b		43.0	310	82.0	Paine (1956), Roback (1957)
Microtendipes sp.		22.0	4.0	0.0	8.5	1.0				Jones (1949)
Paratendipes albimanus (Meigen)	26.2						43.0	310	82.0	Lindeberg (1958), Paine (1956), Roback (1957)
Paratendipes sp.										Brues (1928)
Polypedilum fallax (Johannsen)	26.2						43.0	310	82.0	Paine (1956), Roback (1957)
Polypedilum halterale (Coquillett)					b					Roback (1957)
Polypedilum illinoense (Malloch)	26.2						43.0	310	82.0	Paine (1956), Roback (1957), Wurtz (1960)
Polypedilum nubeculosum (Meigen)					b					Palmen (1956)
Polypedilum simulans Townes										Guyer (1956)
Polypedilum sp.										Teter (1960)
Tanytarsus devinctus (Say)										Paine (1956), Roback (1957)
Tanytarsus jucundus (Walker)										Roback (1957)
Tanytarsus nigricans (Johannsen)	26.2						43.0	310	82.0	Paine (1956), Roback (1957)
Tanytarsus varius Townes	26.2						43.0	310	82.0	Paine (1956)
Tanytarsus sp.		22.0	4.0	0.0	8.5	1.0				Jones (1949)
Xenochironomus scupula Townes	26.2						43.0	310	82.0	Paine (1956)
Cryptochironomus blarina Townes										Curry (1958)
Cryptochironomus digitatus (Malloch)										Curry (1958), Guyer (1956), Lindeberg (1958), Tebo (1955)
Cryptochironomus fulvus (Johannsen)	26.2						43.0	310	82.0	Curry (1958), Paine (1956), Roback (1957)
Tendipes anthracinus (Zetterstedt)					b					Lindeberg (1958)
Tendipes atrovirides Townes					b					Teter (1960), Roback (1957)
Tendipes attenuatus (Walker)	26.2				b		43.0	310	82.0	Dicke (1957), Paine (1956), Lindeberg (1958), Palmen (1956)
Tendipes dux (Johannsen)	26.2						43.0	310	82.0	Paine (1956)
Tendipes fumidus (Johannsen)										Roback (1957)
Tendipes milleri Townes										Roback (1957)
Tendipes modestus (Say)										Roback (1957)
Tendipes neomodestus (Malloch)	26.2						43.0	310	82.0	Paine (1956), Roback (1957)
Tendipes nervosus (Staeger)					b					Guyer (1956), Palmen (1956)
Tendipes ochreatus Townes										

Table 3. RANGES OF ENVIRONMENTAL FACTORS FOR FRESH-WATER TENDIPEDID SPECIES (CONTINUED)

Species	$PO_4^{\equiv}$, ppm, total max	Ca^{++}, ppm, max	Mg^{++}, ppm, max	Fe^{+++}, ppm, max	Cl^-, ppm, max	$SO_4^{=}$, ppm, max	NO_3^-, ppm, total max	$CO_3^{=}$, ppm, max	BOD, max	Authors
Tendipedinae (continued)										
Tendipes paganus (Meigen)										
Tendipes plumosus (Linne)										Dicke (1957)
Tendipes riparius (Meigen)	26.2						43.0	310	82.0	Paine (1956), Roback (1957), Wurtz (1960)
Tendipes staegeri (Lundbeck)										Roback (1957)
Tendipes tendipediformis (Goetghebuer)										
Tendipes tentans (Fabricius)					b					Brues (1928), Lindeberg (1958)
Tendipes tuxis (Curran)										
Tendipes sp.		22.0	4.0	0.0	8.5	1.0				Jones (1959)
Glyptotendipes barbipes (Staeger)					b					Lindeberg (1958)
Glyptotendipes lobiferus	26.2						43.0	310	82.0	Paine (1956)
Glyptotendipes paripes (Edwards)					b					Guyer (1956)
Glyptotendipes sp.	26.2						43.0	310	82.0	Dicke (1957), Paine (1956), Wurtz (1960)
Harnischia tenuicaudata (Malloch)										

[a] tr = trace.

[b] Six percent sea water.

Field data indicate that some of these larvae have made suitable adaptations and have now become established in these environments.

Wurtz and Bridges (1961) have reported bioassay studies conducted on several invertebrate forms, including *T. attenautus*, using solutions of zinc and copper sulfate. At present, the results are inconclusive.

Biological Oxygen Demand. Unfortunately, there are very few recorded data that can be used for comparative purposes. In many instances, data could not be used because of incomplete classification of the tendipedids (Table 3). Paine and Gaufin (1956) have reported that many of the genera of the family are able to survive in a BOD of 82.0 ppm. Here, as in the case of ionic concentration, bioassays must be run on selected species as described by Henderson (1957).

Bottom type and siltation. No report was found discussing the various organic or inorganic settleable solids per se. Habitat requirements were listed only in general terms of environmental conditions (Table 4). The Aquatic Life Advisory Committee (1956) established a rating in which organic or inorganic settleable solids affected production in different ways. The presence of organisms will depend upon the amount of settleable solids in the water. Based upon a population rating, the various substrata are rated as follows: Sand, 1; marl, 6; fine gravel, 9; sand and silt, 10.5; gravel and sand, 12; gravel and rubble, 53; aquatic moss on fine gravel, 89; and *Elodea*, 452.

There are many studies in which a decrease in population is illustrated by siltation, sludge, hair and fiber deposits, and scums. No attempt was made, however, to evaluate numerically the amount of settleable solids with the bottom fauna, other than the above rating. Studies on *T. plumosus* and *T. attenuatus* populations in two Michigan lakes indicate that a compact, homogeneous silt is more productive than either clay, marl, or sand. In the case of clay, 8 to 12 inches of turbid water was superimposed over the hydrosol.

CONCLUSION

This survey of the tendiped fauna of fresh-water lakes and streams indicates that several species are adaptable to a wide range of habitat requirements. The species *Tendipes attenuatus*, *T. riparius*, *T. staegeri*, and a species of *Glyptotendipes* are frequently found in polluted areas. Of these forms, *T. attenuatus* seems to be the most adaptable. Possible reasons that this form is able to endure these conditions may be: (1) It is adjusted to a wide range of temperatures; (2) it is adapted to a wide range of hydrogen ion concentration; (3) it is adaptable to anaerobic conditions; (4) it can tolerate high tensions of CO_2; (5) it has the ability to adapt to high ion concentrations as illustrated by salt water habitats; and (6) it is able to adapt to a variety of hydrosols.

The presence of compounds such as sulfides, mercaptans, and resin acid soaps has been shown to be detrimental to bottom fauna (Wilson, 1953). Research involving bioassays with respect to factors in the environment will give additional information regarding "levels of tolerance." Continued field observations will supplement the results observed in the laboratory by conditions found in nature.

Table 4. RANGES OF ENVIRONMENTAL FACTORS FOR FRESH-WATER TENDIPEDID SPECIES

Species	Bottom type	Depth in meters	Lotic	Lenitic	Water[a]			Authors
					P	R	C	
Pelopiinae								
Pentaneura carnea (Fabricius)	gravel, sand, limestone	0.3 to 0.6		X			F	Paine (1956), Roback (1957)
Pentaneura illinoensis (Malloch)	limestone	0.3 to 1.8		X	C	C	C	Paine (1956)
Pentaneura melanops (Weidemann)	gravel, sand, limestone	0.3 to 1.8		X	A	A	A	Paine (1956)
Pentaneura monilis (Linné)	marl, plants	5.0		X				Brues (1922),Roback (1957)
Pentaneura vitellina (Kieffer)	gravel	0.3 to 0.6		X			F	Paine (1956)
Pentaneura sp.	silt, sand, marl, detritus, plants	5.0	X	X				Jones (1948), Wurtz (1960)
Anatopynia dyari (Coquillett)	shale, gravel, sand	0.3 to 1.8		X	A	A	A	Paine (1956), Roback (1957)
Anatopynia sp.	Chara, silt							Jones (1949)
Pelopia punctipennis (Meigen)	shale, gravel, sand	0.3 to 0.6		X		F	F	Paine (1956)
Pelopia stellata (Coquillett)	shale, gravel, sand	1.8		X	A	A	A	Guyer (1956), Paine (1956)
Pelopia vilipennis (Kieffer)	marl, plants	8.2	X					
Pelopia sp.								Brues (1928)
Procladius bellus Loew	pulpy peat, silt	22.0	X					Guyer (1956)
Procladius choreus Meigen	pulpy peat, silt	22.0	X					
Procladius culciformis (Linné)	gravel, shale	0.3 to 0.8		X		F	F	Paine (1956), Roback (1957)
Procladius sp.	gravel, marl	25.0	X	X	A	A	A	Dicke (1957), Jones (1949), Lindeberg (1958), Paine (1956), Teter (1960)
Clinotanypus caliginosus (Johannsen)	gravel, sand	0.3 to 0.6		X			F	Paine (1956)
Coelatanypus concinnus (Coquillett)	gravel, sand, limestone	0.3 to 1.8		X		A	A	Paine (1956)
Diamesinae								
Prodiamesa olivacea (Meigen)	detritus, silt, sand	0.6	X	X			F	Jones (1948)
Syndiamesa sp.								
Diamesa nivoriunda Fitch	gravel, sand, limestone	0.3 to 0.6		X			F	Paine (1956)
Hydrobaeninae								
Corynoneura scutallata (Winnertz)	gravel, sand	0.3 to 0.6		X			F	Paine (1956)
Corynoneura sp.	gravel, sand	0.3 to 0.6		X			F	Jones (1948), Paine (1956), Lindeberg (1958)
Cricotopus absurdus (Johannsen)	limestone	0.3 to 0.6		X			F	Paine (1956)

Table 4. RANGES OF ENVIRONMENTAL FACTORS FOR FRESH-WATER TENDIPEDID SPECIES (CONTINUED)

Species	Bottom type	Depth in meters	Lotic	Lenitic	Water [a]			Authors
					P	R	C	
Hydrobaeninae (continued)								
Cricotopus bicinctus (Meigen)	gravel, sand	0.3 to 0.6		X			C	Baker (1918), Roback (1957), Paine (1956), Wurtz (1960)
Cricotopus exilis (Johannsen)	limestone	0.3 to 1.8		X			A	Paine (1956)
Cricotopus politus (Coquillett)	gravel, sand, limestone	0.3 to 1.8		X			A	Paine (1956)
Cricotopus tricinctus (Meigen)	gravel, sand, limestone	0.3 to 1.8		X			A	Paine (1956)
Cricotopus trifasciatus (Panzer)	gravel, sand	0.3 to 0.6		X			C	Paine (1956), Roback (1957), Wurtz (1960)
Cricotopus sp.								Brues (1928), Jones (1948) (1949)
Hydrobaenus sp.	gravel, marl, plants, sand	25.0	X	X			F	Brues (1928), Paine (1956), Jones (1948) (1949)
Tendipedinae								
Calopsectra dives (Johannsen)	pulpy peat, silt	10.0	X				F	Teter (1960)
Calopsectra johannseni (Bause)	shale	0.3 to 0.8		X			C	Paine (1956)
Calopsectra neoflavella (Malloch)	gravel, sand, shale, till	0.3 to 1.8		X		C	C	Paine (1956)
Calopsectra nigripilus (Johannsen)	gravel, sand, shale	0.3 to 1.8		X			C	Paine (1956)
Calopsectra sp.	marl, plants, sand, shale, gravel	25.0	X	X			C	Dicke (1957), Paine (1956), Teter (1960)
Pseudochironomus richardsoni (Malloch)	sand, till	0.3 to 0.8		X			F	Paine (1956)
Pseudochironomus sp.	plants, marl, silt, pulpy peat	4.0	X				F	
Lauterborniella gracilenta				X			F	Jones (1948)
Lauterborniella varipennis (Coquillett)	marl, plants limestone			X				
Microtendipes pedellus (DeGeer)	detritus, silt, marl, plants, sand	10.0		X			C	Paine (1956), Roback (1957)
Microtendipes sp.	marl, sand, silt, detritus, plants	10.0	X	X			F	Jones (1949)
Paratendipes albimanus (Meigen)	marl, plants, till	10.0		X			C	Lindeberg (1958), Paine (1956 (1956), Roback (1957)
Paratendipes sp.								Brues (1928)
Polypedilum fallax (Johannsen)	gravel, till	0.3 to 0.8		X		C	C	Paine (1956), Roback (1957)
Polypedilum halterale (Coquillett)	plants, marl	4.0	X	X			F	Roback (1957)
Polypedilum illinoense (Malloch)	clay, sand, shale, pulpy peat, gravel	0.3 to 0.8		X			C	Paine (1956), Roback (1957), Wurtz (1960)
Polypedilum nubeculosum (Meigen)	marl, plants	5.0	X				F	Palmen (1956)
Polypedilum simulans Townes	marl, plants	10.0	X				F	Guyer (1956)
Polypedilum sp.	marl, silt, plants, fibrous peat	25.0	X				F	Teter (1960)
Tanytarsus devinctus (Say)	marl, plants	10.0	X	X			F	Paine (1956), Roback (1957)
Tanytarsus jucundus (Walker)	marl, plants	22.0	X	X			F	Roback (1957)
Tanytarsus nigricans (Johannsen)	marl, plants, till, gravel, limestone	10.0	X	X			C	Paine (1956), Roback (1957)

Table 4. RANGES OF ENVIRONMENTAL FACTORS FOR FRESH-WATER TENDIPEDID SPECIES (CONTINUED)

Species	Bottom type	Depth in meters	Lotic	Lenitic	Water[a]			Authors
					P	R	C	
Tendipedinae (continued)								
Tanytarsus varius Townes	sand, till, gravel	00.3 to 0.6		X				Paine (1956)
Tanytarsus sp.								Jones (1949)
Xenochironomus scopula Townes	limestone, till	0.3 to 0.6		X			C	Paine (1956)
Cryptochironomus blarina Townes	marl, clay, silt, pulpy peat, shells	10.0	X				F	Curry (1958)
Cryptochironomus digitatus (Malloch)	rocks, sand, silt, pulpy peat, gravel	6.0	X				F	Curry (1958), Guyer (1956), Lindeberg (1958), Tebo (1955)
Cryptochironomus fulvus (Johannsen)	marl, plants, sand, silt,clay	10.0		X			F	Curry (1958), Paine (1956), Roback (1957)
Tendipes anthracinus (Zetterstedt)	silt, algae	9.0	X				F	Lindeberg (1958)
Tendipes atroviridis Townes	silt, sand, marl, detritus plants	4.0	X				F	Teter (1960), Roback (1957)
Tendipes attenuatus (Walker)	silt, sand, marl, gravel, detritus, plants	22.0	X	X	A	A	A	Dicke (1957), Paine (1956), Lindeberg (1958), Palmén (1956)
Tendipes dux (Johannsen)	gravel, sand, marl, plants	8.0	X	X			F	Paine (1956)
Tendipes fumidus (Johannsen)	marl, plants	5.0	X				F	Roback (1957)
Tendipes milleri Townes	marl, plants	5.0						Roback (1957)
Tendipes modestus (Say)	marl, plants	5.0						Roback (1957)
Tendipes neomodestus (Malloch)	marl, plants, sand, gravel, limestone	5.0		X			F	Paine (1956), Roback (1957)
Tendipes nervosus (Staeger)	marl, plants	8.2	X				C	Guyer (1956), Palmén (1956)
Tendipes ochreatus Townes	marl, plants	4.0	X				F	
Tendipes paganus (Meigen)	sand, silt, marl, detritus, plants	10.0	X				F	
Tendipes plumosus (Linne)	plants, silt, marl, pulpy peat, clay	22.0	X	X			A	Dicke (1957)
Tendipes riparius (Meigen)	gravel, limestone, marl, plants, silt	8.2	X	X	C	C	C	Paine (1956), Roback (1957), Wurtz (1960)
Tendipes staegeri (Lundbeck)	plants, marl, silt, pulpy peat	8.2	X	X		C	C	Roback (1957)
Tendipes tendipediformis (Goetghebuer)	marl, plants	8.2	X				F	
Tendipes tentans (Fabricius)	silt, plants, marl	8.2	X				F	Brues (1928), Lindeberg (1958)
Tendipes tuxis (Curran)	sand, marl, plants, silt, pulpy peat	5.0						
Tendipes sp.	silt, marl, plants, pulpy peat							Jones (1949)
Glyptotendipes barbipes (Staeger)								Lindeberg (1958)
Glyptotendipes lobiferus (Say)	marl, plants	5.0		X			F	Paine (1956)
Glyptotendipes paripes (Edwards)	marl, plants, sand, peat	5.0		X			F	Guyer (1956), Warnhoff (1957)
Glyptotendipes sp.	pulpy peat, till, silt, sand	10.0		X	A	A	F	Dicke (1957), Paine (1956), Wurtz (1960)
Harnischia tenuicaudata (Malloch)	marl, plants, pulpy peat, silt	10.0						

[a] Water P R C = P = pollutional R = recovery from pollution C = clean
F = few C = common A = abundant

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THE INFLUENCE OF PREDATION ON THE COMPOSITION OF FRESH-WATER COMMUNITIES

*T. T. Macan**

I have data on the effects of predation in a moorland fishpond and in a small stony stream. The invertebrate fauna of the fishpond was studied for 5 years, during which the water was kept free of fish, except for a few eels. Five hundred *Salmo trutta* were introduced in the autumn of 1960 and the same number a year later, which, since the pond is but about half a hectare (1 acre) in extent, should have produced a considerable predation pressure. Tadpoles of *Rana* and *Bufo*, which have no reaction that would keep them concealed from predators, disappeared at once; so did the surface-feeding *Notonecta*. The numbers of other animals have so far changed little. Presumably the trout catch only those specimens that wander out of their normal habitat, in which some other factor is controlling the density of population. The most abundant animals are the nymphs of two species of Zygoptera (Odonata) and of *Leptophlebia* spp. (Ephemeroptera). One-year dragonfly nymphs were much more abundant than usual. This did not, as far as could be seen, lead to increased cannibalism by older nymphs nor to the death of a high proportion of the population from starvation. It was observed, however, that, at the end of the second summer, when normally all nymphs are nearly full-grown, only a proportion had reached this length and the rest had grown very little. This suggests that the size of the population is limited by the number of territories in which nymphs can procure enough to eat to grow at the usual rate. That is not, however, the line I wish to follow today.

The stony stream was affected by the overloading of a septic tank, and a great increase in the number of planarians is thought to have been connected with the increased amount of food contributed by the organic matter washed out of it. The total numbers of *Polycelis felina* taken in 1950, 1951, and 1952 were respectively 2, 2, and 6, per square metre, whereas comparable collections since the overloading have yielded up to 1,000 specimens. Certain species of Ephemeroptera and Plecoptera have gradually become scarcer and some have disappeared. This is attributed to predation by the planarian. Had it been owing to pollution, the effect would have been more sudden and would have been manifest sooner. I gave a full account of these results at a symposium on Running Water held in Lucerne last year and organized by Societas Internationalis Limnologiae. It is not necessary to repeat the details here. I wish now to consider certain wider implications of the findings.

Predation on insects by other invertebrates may be an important factor in the characteristic differences between the littoral faunas of productive and unproductive lakes. Table 1 presents data about those groups that are well represented by species that can be identified in two lakes: Esrom, a rich lowland lake in Denmark, and Windermere, which lies in the poorer soils of the English Lake District. There are about 10 times more animals per square metre in Esrom than in Windermere, but the point to be underlined in the present context is that the composition of

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Table 1. COMPARISON OF LITTORAL FAUNA OF ESROM LAKE (BERG 1938) AND WINDERMERE (MOON 1934)

	No. of spp.	Indiv/m^2 at 0.2m	%	No. of spp.	Indiv/m^2 on rocky shore	%
Mollusca [a]	14	1,410	36	9	120 [d]	30
Amphipoda	2	768	19	1	37	9
Isopoda	1	548	14	2	5	1
Hirudinea	10	454	12	6	1	1
Oligochaeta	14	304	3	=4+[c]	17	4
Planarians	6	2+		5	3	1
			89			45
Trichoptera	11	338	8	18+[c]	217	50
Ephemeroptera [b].	1	20	1	6	12	3
Plecoptera	1	76	2	6	5	1
		3,920	11		417	54

[a] Snails only.
[b] Excluding burrowers.
[c] Identifications uncertain.
[d] Ninety were *Ancylus fluviatilis*, which is not recorded in Esrom Lake.

the fauna is markedly different in the two lakes. Berg found only one species of *Asellus* in Esrom, whereas two have been recorded in Windermere, but every other group that does not belong to the Insecta is represented by more species in Esrom than in Windermere. The converse is true of the insect groups. Windermere does not represent an extreme type by any means, being one of the most productive lakes in the English Lake District. These can be arranged in a series (Pearsall, 1921). At the other extreme lie oligotrophic lakes such as Ennerdale and Wastwater. None has been surveyed, but it is known that only two species of gastropod (Macan, 1950) and no planarians (Reynoldson, personal communication) occur in Ennerdale. *Asellus* has not been recorded from it, and the number of species of Hirudinea will certainly be smaller, to judge from what is known of their ecology elsewhere (Mann, 1955). The proportion of insects in its fauna is probably distinctly bigger than in Windermere.

Many of the groups that are so well represented in the rich lake came to fresh water from the sea. It is possible that some have not been able to colonize poor lakes because they cannot take up from the waters of such places all the ions they require. Calcium is one element that is frequently limiting, and the number of species of snail (Boycott, 1936), leech (Mann, 1955), and flatworm (Reynoldson, 1958) has been shown to decrease in a series of waters in which the concentration of calcium is falling. It is also likely that decreasing food supply plays a part, or the more unproductive lakes may lack some growth factor of the type postulated by Lund (Mackereth, Lund, and Macan, 1957).

Insects have invaded fresh water after having successfully colonized the land, where their survival depended on an impermeable cuticle that prevented drying up, and on their ability to obtain everything they required from their food. These properties enabled them to invade waters with a very low concentration of dissolved substances. Their relative scarcity in richer places cannot be owing to anything unfavourable about the water or the substances dissolved in it, though there is a possibility that oxygen could be important. Unfortunately, insufficient samples have been taken at the time when a critical low value is likely to be reached, that is, just before dawn. I postulate that their scarcity is related to the abundance of the other animals, which eat them just as the planarians in the stream ate them. Biotic influences, then, play an important part in determining the structure of the communities inhabiting different kinds of lakes.

If an animal is to survive among many other animals, some method of protecting its eggs is probably of the highest importance. This is lacking in Ephemeroptera and Plecoptera. Among the former, a few species of *Baetis* crawl into the water and glue their eggs to a stone, but most wash a clump of eggs off the abdomen by dipping the tip of it into the water. The eggs then fly apart and fall to the bottom. The eggs of Plecoptera are dispersed in the same way, when a female swims across the water surface. Many must come to rest in the algal felt covering the surface of stones, where they will be eaten incidentally by any algal browser such as a snail. Others, falling between the stones, may land in accumulations of debris and in due course may be devoured by some detritus feeder. Most species lay many eggs and so the nymphs are small. Frequently they emerge in autumn and grow slowly, and spend, therefore a long time in the small, vulnerable stage.

In contrast, the animals typical of rich waters produce their eggs in such a way that there is much less danger that the eggs will be eaten. The snails lay a number together in a gelatinous envelope too large to be eaten incidentally by a browser or by most carnivores. Leeches and flatworms lay their eggs in cocoons. *Gammarus* and *Asellus* keep theirs in a brood pouch until the young hatch, and they are safe from everything except animals large enough to devour the brooding parent. All these groups lay fewer eggs than the insects mentioned and generally bring them forth at a time of year when conditions for growth are optimum. The young are, therefore, larger on emergence and grow quickly, so that they are in danger from all but the largest carnivores for a short period only.

At present there is a good deal of speculation about this hypothesis. If it proves to be well founded, it will add a complicating factor to any "Saprobien-system," for these systems seem to make insufficient allowance for the effects of species on each other.

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DISCUSSION

(GAUFIN)

Comments following Dr. Gaufin's paper included one to the effect that it is probably the eggs and not young stoneflies that survive over periods of drought. Also in certain species, it is during the egg stage (a fairly extensive period) that low oxygen concentrations possibly have the most detrimental effect, because nymphs can shift their own location for a more adequate supply of oxygen.

Older nymphs of 68 species have been observed to begin and increase their "pumping" or "pushup" rate as the dissolved-oxygen level was reduced. When the level reached a certain low point, the pumping ceased and the animals became relatively inactive, as though conserving energy.

Concerning toxicity of pesticides, such compounds as DDT, aldrin, endrin, heptachlor, dieldrin, fishtox, and others have been used in static and continuous-flowthrough experiments on stonefly nymphs, and the general indication is that they are more sensitive than most fishes.

Relative toxicity of several pollutants to fish, caddisfly larvae, stonefly nymphs, and stonefly eggs was discussed at some length. In a pollution case in Africa the toxic substance killed many stonefly nymphs, but not their eggs or fish that were in the same stream. In another such instance in Britain an unknown quantity of BHC killed many stonefly and caddisfly species with no apparent kill of fishes.

Lab studies indicate that a species of *Gammarus* is just as sensitive to some insecticides as the stoneflies, and that certain caddisflies *(Hydropsyche* and *Arctopsyche)* are less sensitive.

A final item of interest involved stonefly life cycles. *Pteronarcys californica* has a 3-year cycle; *Acroneuria pacifica, A. media,* and *Pteronarcella badia* a 2-year cycle; and *Alloperla, Isoperla, Nemoura,* and *Taeniopteryx*, etc., all have a 1-year cycle.

(LEONARD)

An informative exchange of observations on several species of mayflies followed Dr. Leonard's paper. In British streams, *Heptagenia* seems limited to water temperatures of 18° C or less. In Irish limestone lakes, one species of this genus occurs abundantly, but another species dominates the limed-fissured areas around the edge of Ireland. In Michigan, *Stenonema* is common in streams flowing through glacial drift, but in waters that flow over Pre-Cambrian bedrock, *Heptagenia* is the common mayfly.

Studies in Switzerland indicated that one mayfly, *Rhithrogena*, the gills of which serve as a sucker attachment to the substrata, shows an increase in oxygen consumption with increasing water current. The lethal concentration decreases as water current increases. *Heptagenia*, the gills of which can be used to cause a current of water to flow over the body, shows little or no change in oxygen consumption with increased water flow. As a possible consequence, the first species seems limited to running water, whereas the latter is found in lakes and streams. In another study, oxygen consumption apparently increased as dissolved-oxygen concentration increased; the investigators thought this might be due to some experimental artifact.

Another interpretation of the Switzerland work was that the metabolic rates were depressed at low-flow low-oxygen levels and that when the flow increased, the metabolic rates merely returned to normal (higher) levels.

A question for future consideration was then put to the audience: Is the maximal activity dependent on the water flow (bringing more oxygen) or merely indicative of the conditions persisting at that time?

(ROBACK)

Discussion of Dr. Roback's paper included several comments concerning the high carbon dioxide levels and the significance of BOD readings alone. One investigator reported on a stream polluted by heavy metal wastes. The caddisflies "stayed put" when mayflies disappeared and fish populations were somewhat reduced.

(CURRY)

During the discussion of Dr. Curry's paper, he described a tank that is being used to study the effects of anaerobic conditions on midge larvae. It is stainless steel, portholes and cover are of plexiglas, and it is built to withstand 15 atmospheres of pressure. Methane, carbon dioxide, hydrogen sulfide, or other gases can be bubbled into the closed tank containing substratum and animals in their original water. The portholes allow observation. Careful handling (as with other test animals) appears extremely important.

(MACAN)

In the discussion of Dr. Macan's paper the possible reasons for the upsurge of planarians were explored. It was suggested that perhaps many small planarians that usually die off were sustained by the addition of sewage fauna and flora. These then gave rise to a large population of adult planarians that possibly were able to prey upon some of the insect larvae.

A comment was made to the effect that most generally in terrestrial situations, predator populations rise and fall as a result of prey population changes, rather than cause such changes. In aquatic situations, often the abundance of predators seems to have a great effect on the prey numbers. In one illustration comparing three similar southern ponds, one with bass only had a large number of bottom organisms, another with bass and bluegills had only a sparse bottom fauna, and a third, greatly overpopulated with bluegills, had virtually no typical midges or other aquatic insects.

In many pollution situations, a common occurrence seems to be that when predators are killed off, prey populations develop in vast numbers.

In some northern thermally stratified lakes managed by trout stocking, one possible reason for the decrease in the general condition of these fish after 3 to 6 years is that they clean out the readily available bottom fauna and simply do not get enough food.

ENVIRONMENTAL REQUIREMENTS OF FISHES AND WILDLIFE

F.T.K. Pentelow, Chairman (Fishes) [*]
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DISSOLVED OXYGEN REQUIREMENTS OF FISHES [‡]

Peter Doudoroff [§] *and Charles E. Warren* [//]

INTRODUCTION

Minimum dissolved oxygen concentrations that can be tolerated by different fishes for limited periods of time have been determined in various ways by a number of investigators. The results of most of these studies have been briefly but adequately summarized elsewhere (Doudoroff, 1957; Tarzwell, 1957; Townsend and Earnest, 1940; Ellis, 1937). Doudoroff (1957) concluded that, in the absence of other harmful conditions or agents, "mortality of fishes at concentrations (of dissolved oxygen) near and above 3 ppm is not to be expected ordinarily, even when the concentrations persist for long periods. Concentrations in the neighborhood of 2 ppm often may be critical for sensitive forms. Yet, at moderately low temperatures and under otherwise favorable conditions, concentrations near and below 1 ppm evidently can be tolerated for long periods not only by the most resistant species, but also by more susceptible fishes which have been thoroughly acclimatized to low dissolved oxygen concentrations." Tolerance is not, of course, considered here as equivalent to the ability to survive and thrive indefinitely in a natural environment under the specified conditions.

Apparently, for reasons discussed by Tarzwell (1957) and others, it is now generally realized that fishes cannot be expected to thrive in their natural habitat at barely nonlethal oxygen concentrations. Dissolved oxygen concentrations below 3 milligrams per liter (or ppm) in waters inhabited by salmonids or other sensitive and valuable fish species, and concentrations below 2 milligrams per liter in waters inhabited only by relatively tolerant species of value probably are not deemed satisfactory or acceptable by anyone charged with water pollution prevention and control. Higher concentration levels now seem to be generally prescribed by regulatory agencies that have adopted specific standards applicable to waters in which thriving fish populations are to be fully protected.

Fishes have been found to grow little or not at all, and even to lose weight in the presence of an abundant supply of food, at reduced oxygen concentrations that could be tolerated indefinitely or for long periods of time (Davison et al., 1959; Herrmann, Warren, and Doudoroff, 1962; Stewart, 1962). Swimming performance also has been found to be impaired at such concentrations (Fry, 1957; Katz, Pritchard, and Warren, 1959; Davis et al., 1963). Salmonid larvae newly hatched after embryonic development at constant low oxygen concentrations that did not demonstrably influence the survival of the embryos or hatching success have been found to be exceedingly small and weak (Silver, 1960; Shumway, 1960).

The sublethal adverse effects of reduced oxygen concentrations have not until recently been studied intensively, however, and they clearly deserve more attention than they have received in the past. Some of these effects can be precisely measured and related to their true causes only through laboratory experimentation. The difficulty of estimating the ecological significance of effects observed under laboratory conditions should not be regarded (as it seems to have been by Moss and Scott, 1961) as a valid justification for continued emphasis on the determination of oxygen concentrations that are barely tolerable for resting and fasting fish, and at which normal development, growth, and activity are impossible. The obstacles to reliable interpretation of the experimental data with reference to natural or field conditions, which is essential to sound practical application of the data, must be overcome through intensified effort.

In this paper, the available information on the nonlethal effects on fresh-water fishes of reduced oxygen concentrations, of abnormally high concentrations and of large diurnal fluctuation of concentration is reviewed briefly, with emphasis on the most recent advances of knowledge based on laboratory experiments. In the concluding discussion, some possible new ways of arriving on ecologically sound conclusions concerning the dissolved oxygen requirements and other water quality requirements of fishes through laboratory experimentation are suggested, after consideration of some of the interrelations and the probable ecological significance of the various kinds of data summarized. It is realized that an attempt to interpret or synthesize fully the available knowledge would be premature, for essential information of various kinds still is lacking and much of it can be expected to become available soon. It is hoped, however, that by focusing attention on some of the major unsolved problems the concluding discussion will be helpful in stimulating and guiding into profitable channels some of the needed additional research.

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EFFECTS OF VARIATIONS OF DISSOLVED OXYGEN

EFFECTS ON OXYGEN CONSUMPTION

Data on the influence of oxygen concentration on the oxygen uptake rates of fish have been recently reviewed and discussed by Fry (1957) and by Winberg (1960) and so will not be considered in detail here.

The oxygen consumption rate of undisturbed, unfed, and mostly resting fish cannot be much reduced through suspension of any unessential muscular activity. Therefore, a reduction of oxygen concentration to a level that is not nearly sufficient for the maintenance of this resting or "standard" uptake rate can be expected to result in interference with essential metabolic processes. Death of the fish can be expected to follow soon, until life can be indefinitely sustained at the low oxygen concentrations largely or entirely by anaerobic metabolism, as it has been reported to be sustained at low temperatures in some fish (Blazka, 1958). Moss and Scott (1961) found that resting largemouth bass, *Micropterus salmoides*, bluegill, *Lepomis macrochirus*, and channel catfish, *Ictalurus punctatus*, held at 25, 30, or 35°C in a respirometer in which the oxygen concentration was gradually reduced, usually showed no marked reduction of oxygen consumption rates until a lethal oxygen concentration was reached and the fish were about to die.

The rate of oxygen consumption of active brook trout, *Salvelinus fontinalis*, has been found to be dependent on oxygen concentration at concentration levels near or even well above the air-saturation level (Job, 1955; Basu, 1959). When the rate was found to be dependent on oxygen concentration only at much lower levels (e.g., Graham, 1949), the fish may not have been stimulated to maximum activity, or they may have been unhealthy (e.g., anemic). Acclimation of brook trout to reduced oxygen concentrations has been shown to influence the "active" oxygen uptake rated (Shepard, 1955).

Basu (1959) and Fry (1961) have reported little or no increase of oxygen uptake rates of active brook trout and of carp, *Cyprinus carpio*, with threefold to sixfold increases of swiming speed. Recent introduction of the fish into a respirometer could have evoked a maximal oxygen uptake rate at each level of performance. However, Basu reported also that large increases of the oxygen uptake rates of active trout resulted from mild electrical stimulation apparently without any increase of activity (swimming speed). The physiological significance of published data on active oxygen uptake rates of fish and their dependence on oxygen concentration thus is still somewhat obscure. Further clarification thereof is needed before these metabolic rate data can be properly related to data on performance, or used very effectively in evaluating the performance capabilities of fishes at different oxygen concentrations. Fry's (1947, 1957) conclusion that the "cruising speed" of fish increases as the square root of the "metabolic difference" or "scope for activity" (i.e., the active oxygen uptake rate less the standard rate) increases can now be accepted only with some reservations.

EFFECTS ON SWIMMING PERFORMANCE

Davis et al., (1963) found that the maximum sustained swimming speeds of juvenile coho salmon, *Oncorhynchus kisutch*, and chinook salmon *O. tshawytscha*, at temperatures of 10 to 20°C usually declined with any considerable reduction of oxygen concentration from air-saturation level. In other words, these speeds usually were dependent on oxygen concentration at all concentration levels below the saturation level. One lot of chinook salmon, which may have been anemic, was notably exceptional. The performance of these fish at high oxygen concentrations was not markedly better than that observed at concentrations between 5 and 7 milligrams per liter in tests performed at 20°C. Graham (1949) observed a similar relation between oxygen concentration and the cruising speed of brook trout in an experiment with three fish tested in a rotating annular chamber at 8°C.

Figure 1 shows the relation, at each of three different temperatures (10°, 15°, and 20° C), between oxygen concentration and the lowest water velocity that could not be resisted by all individuals in a group of five underyearling coho salmon subjected to gradually increasing velocities (0.08 fps increments at 10-minute intervals) in a tubular experimental chamber. Curves relating oxygen concentrations to water velocities at which swimming failure of the second fish occurred in a group of five were not markedly different in shape from the curves based on first failures shown in Figure 1. It is noteworthy that increases of oxygen concentrations beyond the air-saturation levels had little or no influence on the performance of the salmon.

Recent experiments by M. L. Dahlberg at Oregon State University, in which groups of five juvenile largemouth bass were tested in the tubular chamber at 25°C, have shown the mean maximum sustained swimming speeds of these fish to be virtually independent of oxygen concentration at concentrations above 5 milligrams per liter (Figure 2). The maximum sustained swimming speeds of the first-failing fish in a group of five showed a poorer fit to any curve, but about the same general relation to oxygen concentration.

Ferguson's data reported by Fry (1957) indicate some reduction of the cruising speed of yellow perch, *Perca flavescens*, at reduced oxygen concentrations only a little below the air-saturation level, especially

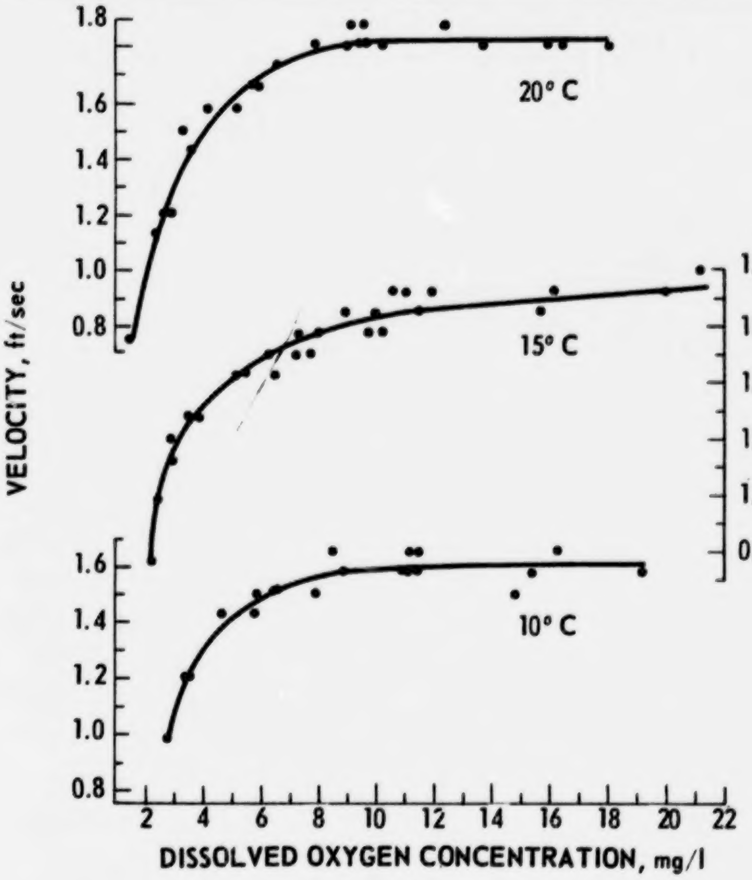

Figure 1. Water velocities at which sustained swimming failures of first-failing wild underyearling coho salmon (in groups of 5 fish) occurred at 10, 15, and 20°C, in relation to oxygen concentration. (After Davis, Warren, and Doudoroff, 1963.)

at the higher test temperatures. The performance of these fish, however, was greatly impaired only at concentrations below 3 milligrams per liter. At about this concentration, there was apparently a rather abrupt change in the slope of curves relating the cruising speed to dissolved oxygen (except at the lowest test temperature of 10°C).

The influence of oxygen concentration on the resistance of fish to fatigue resulting from intense activity ("burst" performance) or from prolonged swimming at speeds approaching the maximum sustained or cruising speed has not yet been adequately investigated. Katz, Pritchard, and Warren (1959) have demonstrated the ability of juvenile coho and chinook salmon, which were tested at 20° C, to swim for 24 or 48 hours at the moderately high speed of 0.8 foot per second at oxygen concentrations near 3 milligrams per liter. These concentrations are not very much above the concentrations that would be lethal to the fish under conditions necessitating no sustained activity. Maximum current velocities that can be resisted for 24 hours or longer at any given oxygen concentration possibly are only slightly less than those that can be resisted only for relatively short periods (e.g., 10 or 20 minutes), and "burst" swimming speeds may not be influenced by oxygen availability as markedly as are the sustained swimming speeds. Some of the relationships in question are now being investigated to evaluate and understand more fully the effects of reduced oxygen concentrations on swimming performance of different kinds, involving different intensities of activity.

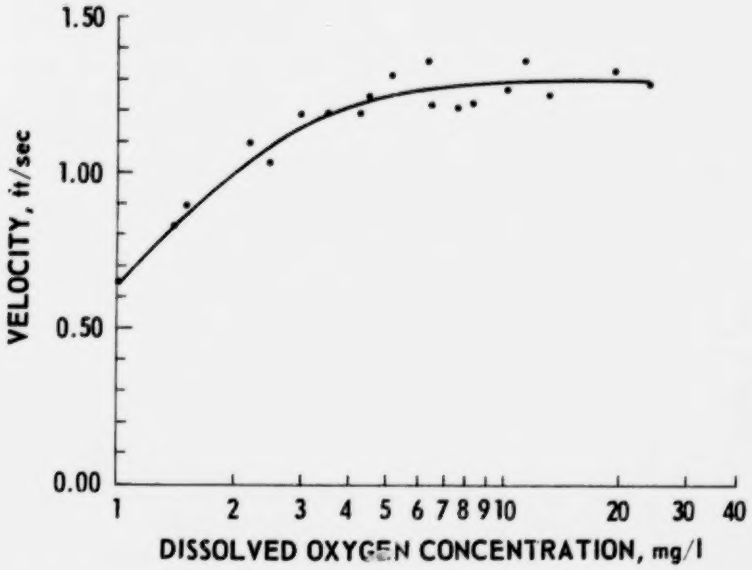

Figure 2. Water velocities (mean) at which sustained swimming failures of juvenile largemouth bass occurred at 25°C, in relation to oxygen concentrations. Each plotted point represents a mean velocity based on observations of 5 fish tested simultaneously at the same oxygen concentration. The velocity at the time of failure of each fish was recorded, and the fish was then removed from the test chamber.

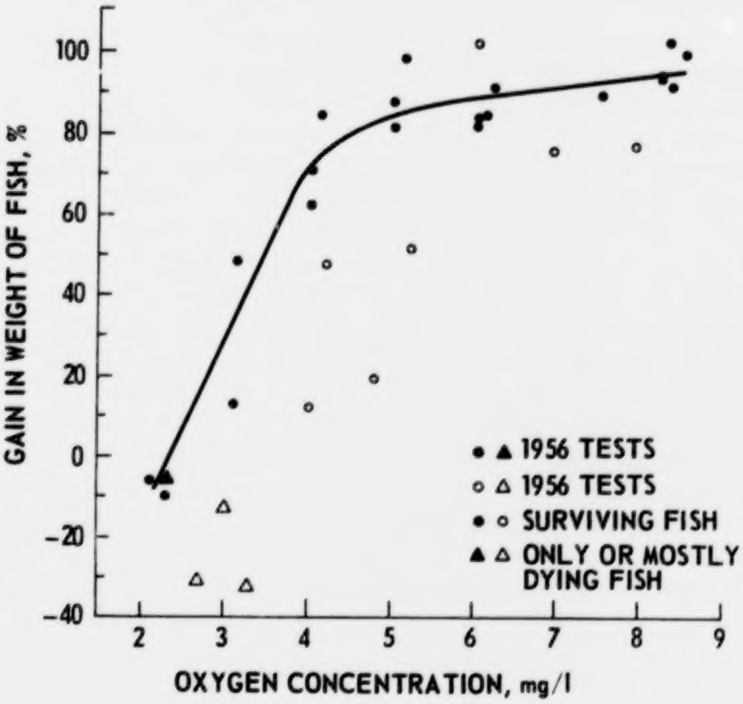

Figure 3. Percent gains (or losses) in wet weight of underyearling coho salmon fed beach hoppers abundantly for 1? to 28 days at 20°C, in relation to dissolved oxygen concentrations. The curve has been fitted to the 1956 data only, since the results of 1955 tests probably were affected by a toxicant from rubber tubing. All of the 1956 positive weight gains shown are results of 21-day tests. (After Herrmann, Warren, and Doudoroff, 1962.)

EFFECTS ON APPETITE AND GROWTH

Hermann, Warren, and Doudoroff (1962) found that the growth rates of abundantly fed underyearling coho salmon, which were kept at 20° C on a diet of beach hoppers (marine amphipods), declined slightly with reduction of oxygen concentration from a mean of about 8.3 milligrams per liter to 6 and 5 milligrams per liter, and declined more sharply with further reduction of oxygen concentration (Figure 3). The rates of food consumption declined likewise; however, the gross efficiency of food conversion was markedly reduced only at oxygen concentrations well below 4 milligrams per liter. Therefore, the depression of growth rates at moderately reduced oxygen concentrations is directly attributable to impairment of appetite. The unimpaired efficiency of utilization for growth of the relatively small amount of food consumed at these concentrations evidently is ascribable to reduction of activity and of maintenance food requirements, or to improvement of digestive and assimilatory efficiency due to reduction of food intake. Juvenile coho salmon consumed very little food and lost weight at oxygen concentrations averaging 2.0 to 2.3 milligrams per liter and at temperatures of 18° to 20°C (Herrmann et al., 1962; Davison et al., 1959). Whether digestive and assimilatory efficiency is or is not materially impaired at the very low nonlethal concentrations has not been established. The resistance of juvenile coho salmon to the lethal and growth-depressing effects of low oxygen concentrations at moderately high, constant temperatures apparently varies somewhat with the season or the age of the fish.

Stewart (1962) found that the growth rates of juvenile largemouth bass kept at temperatures near 26°C on an unrestricted diet of earthworms generally were quite markedly reduced with any considerable reduction of oxygen concentration from the air-saturation level of about 8.2 milligrams per liter (Figure 4). At high oxygen concentrations equal to about two to three times the air-saturation value (i.e., 17.5 to 24 mg/1), the percent weight gains of the bass generally were considerably less than those observed at concentrations near the air-saturation level in the same experiments. As in the experiments with coho salmon (Herrmann et al., 1962), the food consumption rates of the bass varied with oxygen concentration in much the same way as did the observed gains in weight, and gross food conversion efficiencies generally were markedly reduced only at oxygen concentrations below 4 milligrams per liter.

The appetite and growth rates of the bass were greatly impaired by wide diurnal fluctuations of oxygen concentration so timed that the fish were exposed once daily to low concentrations at night and through early morning. This impairment was evident even when the mean oxygen concentrations (i.e., the properly weighted means, arithmetic and geometric, of the alternating high and low concentrations to which the fish were exposed) fell well within the range of favorable constant concentrations, and when the duration of exposure to lower concentrations was only about one-half or even one-third of each 24-hour day. For example, in Experiment No. 4, bass exposed alternately to 2.0 and 17.4 milligrams per liter for nearly equal periods each day (after gradual transition from one concentration to the other) showed a dry weight gain in 15.5 days of only 72 percent. It can be seen from Figure 4 that this weight gain corresponds approximately to that which would have occurred at a constant oxygen concentration of 2.6 milligrams per liter. It is far less than the grain (about) 131 to 135 percent) that would have occurred at constant oxygen concentrations equal to the arithmetic and geometric means of the fluctuating oxygen concentrations (about 10 and 6 mg/1, respectively). Likewise, in Experiment 5, bass exposed to concentrations near 2.0 milligrams per liter for less than one-third of each 24-hour day and to concentrations near 8.1 milligrams per liter for the remainder of the day (excepting periods of transition) showed a dry weight gain in 15 days of only 99 percent. Such a gain would have occured also at a constant oxygen concentration of only about 3.0 milligrams per liter. At constant concentrations equal to the arithmetic and geometric means of the fluctuating concentrations (about 6 and 5 mg/1, respectively), the dry weight gains of the fish used in this experiment would have amounted to about 157 and 142 percent, respectively (Figure 4). It is evident that, even in the absence of lethal concentrations, mean oxygen concentrations in eutrophic waters with wide diurnal fluctuations of dissolved oxygen content are virtually meaningless and can be misleading; growth rates of fish subjected to fluctuating concentrations may be largely dependent on the dissolved oxygen minima occurring at night or early in the morning.

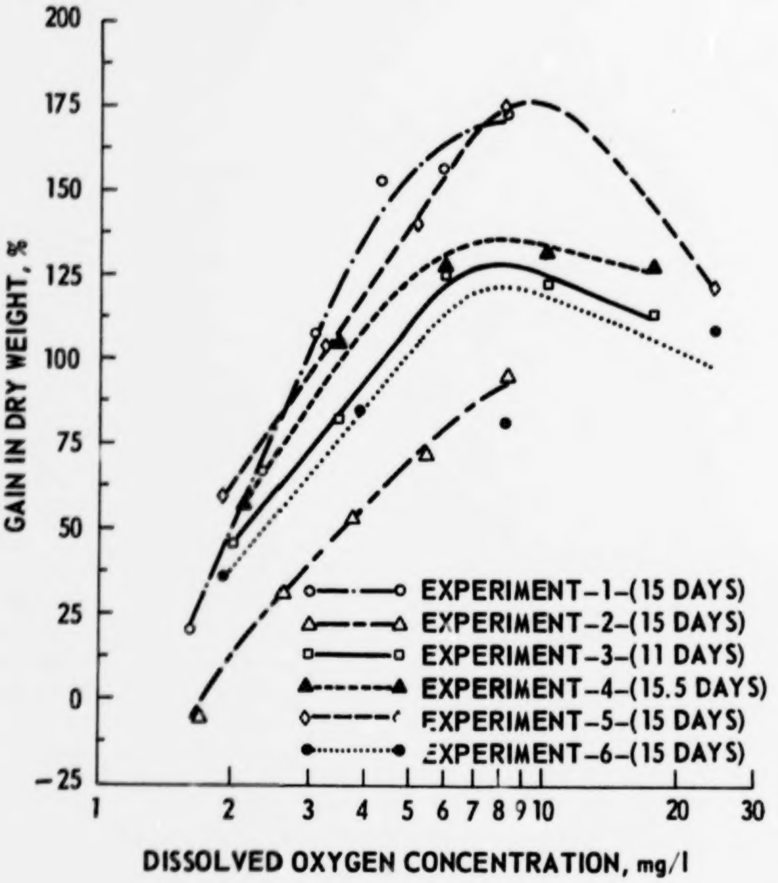

Figure 4. Percent gains in dry weight of juvenile largemouth bass kept on an unrestricted diet of earthworms for 11 to 15.5 days at 26°C, in relation to dissolved oxygen concentrations. (After Stewart, 1962.)

In a further experiment performed recently at Oregon State University by R. J. Fisher, underyearling coho salmon were held at 18°C on an unrestricted diet of tubificid worms (plus some *Daphnia*). The growth rates of the salmon were much greater than those observed at 20°C by Herrmann, Warren, and Doudoroff (1962). At high oxygen concentrations the fish increased in wet weight by about 200 to 220 percent in 18 days. The curve relating the observed percent weight gains to oxygen concentrations below the air-saturation level (Figure 5) is not very different in shape, however, from that based on the earlier experiments (Figure 3). At concentrations near 3 milligrams per liter the wet weight gain in 18 days was only about 108 percent. At concentrations near 18 milligrams per liter, or nearly twice the air-saturation level, the weight gain was slightly greater than that observed at the air-saturation level of oxygen (9.5 mg/1), but at concentrations near 30 milligrams per liter, or more than three times the air-saturation level, the weight gain was slightly less than that observed at the saturation level. Even at the lowest oxygen concentration tested the fish not only grew fairly rapidly, but also showed a gain in relative fat content and consequently a greater percent gain in dry weight than in wet weight (Figure 5). Wide diurnal fluctuations of oxygen concentration involving nightly reductions to low levels were found to be as inimical to the growth of the salmon as to the growth of largemouth bass. Coho salmon exposed alternately to concentrations of 3 and 9.5 milligrams per liter or 3 and 18 milligrams per liter for equal periods each day showed weight gains in 18 days approximately equivalent to the estimated weight gains that would have occurred at constant oxygen concentrations near 3.5 milligrams per liter (Figure 5). The arithmetic or geometric mean oxygen concentrations were all well above 5 milligrams per liter. At constant concentrations equal to these means, growth was nearly as rapid as it was at the apparently nearly optimal constant concentration of about 18 milligrams per liter.

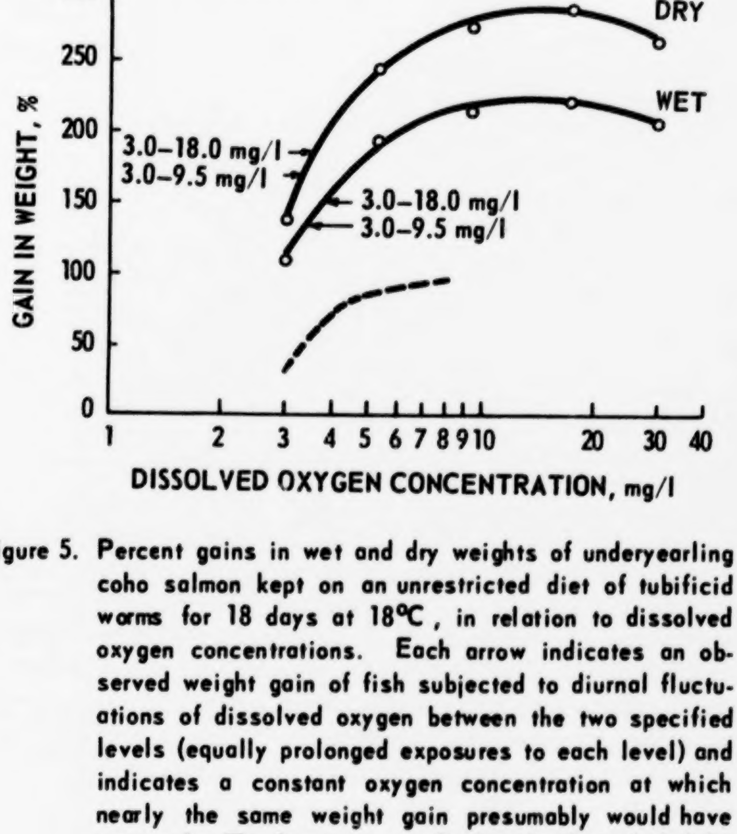

Figure 5. Percent gains in wet and dry weights of underyearling coho salmon kept on an unrestricted diet of tubificid worms for 18 days at 18°C, in relation to dissolved oxygen concentrations. Each arrow indicates an observed weight gain of fish subjected to diurnal fluctuations of dissolved oxygen between the two specified levels (equally prolonged exposures to each level) and indicates a constant oxygen concentration at which nearly the same weight gain presumably would have occurred. The lowest curve (broken line), included for comparative purposes, is based on the data plotted in Figure 3.

EFFECTS ON EMBRYONIC DEVELOPMENT

Salmonid embryos at various developmental stages have been shown in the laboratory to tolerate exposures of limited duration (e.g., 7 days) to dissolved oxygen concentrations well below 2.0 milligrams per liter (Alderdice, Wickett, and Brett, 1958). Development is inhibited or arrested under these conditions, but upon return of the embryos to well-oxygenated water the development can proceed to successful hatching of larvae (i.e., sac fry). Seven-day exposures to even lower oxygen concentrations have resulted in high mortalities of the embryos or in the hatching of structurally defective larvae.

Continued exposure of salmonid embryos to reduced oxygen concentrations well above 2 milligrams per liter may result in high or total embryo mortality or in retardation of embryonic development and delayed hatching of the larvae, which may or may not be deformed (Garside, 1959). High percentages of successful hatching of steelhead trout *(Salmo gairdneri)* and coho and chinook salmon occurred when the embryos were reared from fertilization to hatching in the laboratory at constant temperatures near 10°C (9° to 11°C) and oxygen concentrations as low as 2.5 milligrams per liter (Silver, Warren, and Doudoroff, 1963; Shumway, 1960). Garside (1959) reported that nearly all lake trout embryos perished at the temperature of 10°C when oxygen concentrations averaged 4.2 milligrams per liter, but at lower temperatures many survived to hatching even at oxygen concentrations averaging 2.5 milligrams per liter.

Critical or limiting dissolved oxygen levels, at which the respiratory demand of salmonid embryos is just satisfied and below which the oxygen uptake rates become dependent on oxygen concentration, have been found to vary widely with the age of the embryos. Critical or limiting levels well below the air-saturation levels of dissolved oxygen have been reported for all developmental stages. These values, determined experimentally (Hayes, Wilmot, and Livingstone, 1951) or theoretically (Alderdice, Wickett, and Brett, 1958), are not deemed reliable for various reasons (Silver, Warren, and Doudoroff, 1963). Recent experiments have shown that when salmon and trout embryos are exposed to oxygen concentrations below air-saturation levels throughout the period of their development from fertilization to hatching, not only is the hatching delayed, but also the resulting larvae or sac fry are smaller at hatching than those deriving from embryos reared at high oxygen concentrations (Silver, Warren, and Doudoroff, 1963; Shumway, 1960). The size of the newly-hatched sac fry has been found to be dependent on the oxygen concentration even at concentration levels very near, if not well above, the air-saturation levels at temperatures near 10°C. This dependence is expressed over a wide range of water velocities, on which the rate of development and the size of the larvae at hatching are likewise dependent.

Figure 6 (after Silver, Warren, and Doudoroff, 1963) and Figure 7 (based on unpublished data recently obtained by D. L. Shumway at Oregon State University) show the results of typical experiments in which steelhead trout or coho salmon embryos were subjected throughout development at 9.5° or 10° C to different, nearly constant oxygen concentrations, ranging from less than 3 to about 11 milligrams per liter, at each of four different water velocities, which ranged from 6 or 3 to 740 or 800 centimeters per hour. These embryos were reared in a special apparatus in which oxygen concentration can be controlled independently of the velocity of the water moving upward through porous plates on which the embryos rest (Silver, Warren, and Doudoroff, 1963). The dependence of larval size (at hatching) on oxygen concentration even at high concentration levels and the important influence of water velocity are clearly illustrated in Figures 6 and 7.

It can be concluded that the velocity of movement of water through streambed gravels in which salmonid eggs are deposited must be high enough not only to ensure delivery of enough oxygen to the redds for supplying the total requirements of all the embryos, but also to deliver sufficient oxygen to the surface of the chorion enveloping each individual embryo. Experiments similar to those reported above have been performed recently in which some steelhead trout embryos were embedded in large glass beads, some in small beads, and some in a mixture of these, whereas others were not surrounded by beads and rested separately on the porous plates. Water was recirculated through each of these different artificial redds at equal rates. The availability of oxygen to the embryos at a given oxygen concentration was nevertheless expected to vary with the estimated mean velocity of the water in the voids among the various solid components of the artificial redds (i.e., the mean "pore velocity," which increased as the porosity decreased). The results indicated that the availability of oxygen varied indeed and in a manner generally consistent with the data on the influence of ambient water velocity differences produced without the use of beads by varying the water recirculation or discharge rates only. That increasing the velocity of water around embryos by embedding them in glass beads while maintaining the same water discharge rate can result in an increase of the size of newly hatched coho salmon larvae had been demonstrated earlier by Shumway (1960).

The relative efficiencies of conversion of yolk to embryonic tissue in coho salmon are impaired at moderately low oxygen concentrations and water velocities (Shumway, 1960). However, inasmuch as there is insufficient evidence that, under the same environmental conditions, the yolk conversion efficiency of sac fry also is seriously impaired and there is some evidence that it may not be impaired materially in steelhead trout fry, the importance of the reduction of conversion efficiency observed during embryonic development is still uncertain. Only a small portion of the yolk is utilized during development prior to

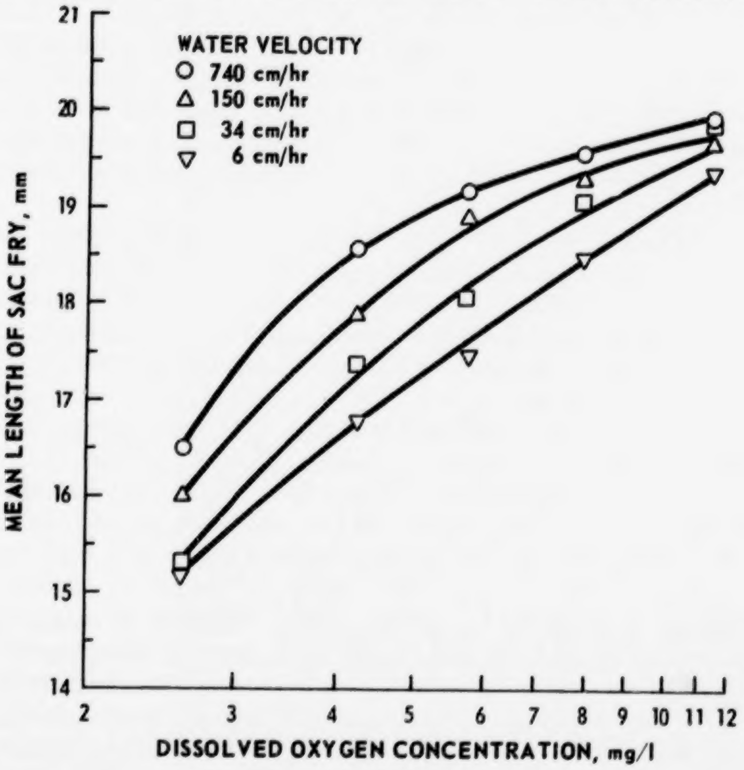

Figure 6. Mean lengths of steelhead trout larvae (sac fry) at the time of their hatching, in relation to oxygen concentrations at which embryos were reared at 9.5°C and at constant water velocities. Each plotted point and curve represents a result obtained at one of four different water velocities tested simultaneously. (After Silver, Warren, and Doudoroff, 1963.)

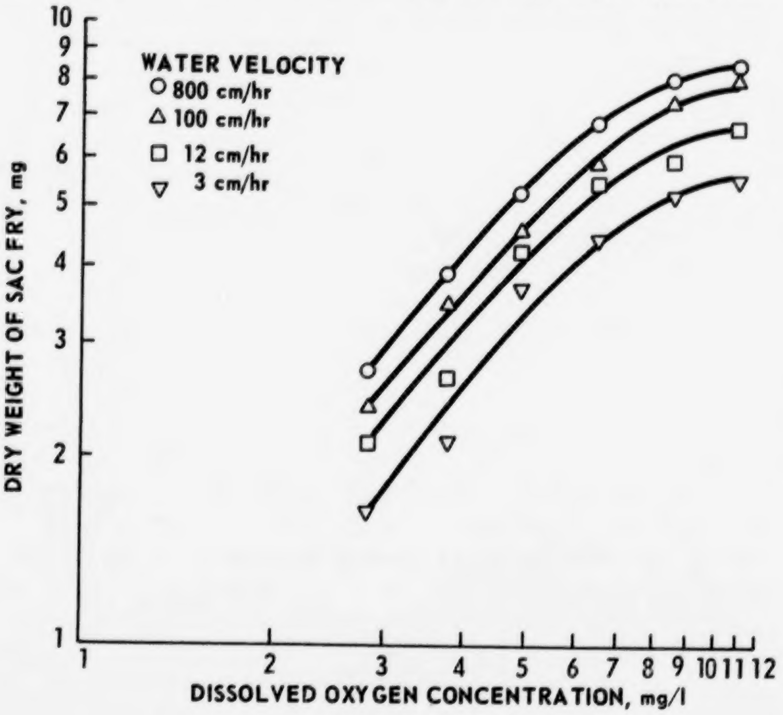

Figure 7. Mean dry weights of coho salmon larvae (sac fry with yolk sacs removed) at the time of their hatching, in relation to oxygen concentrations at which embryos were reared at 10°C and at constant water velocities. Each plotted point and curve represents a result obtained at one of four different water velocities tested simultaneously.

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hatching. Inhibition of the growth of sac fry of Atlantic salmon at reduced oxygen concentrations has been reported by Nikiforov (1952), but the influence of oxygen concentration on the growth of salmonid larvae needs further investigation, with attention to the efficiency of yolk utilization for growth, which efficiency could well be impaired when growth is inhibited.

EFFECTS ON MOVEMENTS (AVOIDANCE REACTIONS)

The ability of fishes to detect and avoid low oxygen concentrations under experimental conditions in the laboratory has been repeatedly demonstrated (Shelford and Allee, 1913; Jones, 1952; Whitmore, Warren, and Doudoroff, 1960; Hoglund, 1961). Whitmore, Warren, and Doudoroff (1960) found that juvenile chinook salmon showed marked and prompt avoidance of reduced oxygen concentrations as high as 4.5 milligrams per liter at summer temperatures. The behavior of coho salmon was somewhat erratic and their avoidance of low oxygen concentrations less pronounced than that of the chinook salmon, but there was apparently some avoidance of concentrations as high as 6.0 milligrams per liter. Juvenile largemouth bass and bluegills markedly avoided concentrations near 1.5 milligrams per liter, but showed little or no avoidance of concentrations near 3.0 and 4.5 milligrams per liter; only the bass showed any avoidance of the latter concentration.

The very prompt avoidance by some of the fish, especially chinook salmon, of reduced oxygen concentrations well above those known to be lethal to the fish or to elicit visible symptoms of acute respiratory distress was not deemed ascribable entirely to mere stimulation or increase of the activity of the fish caused by oxygen deficiency. Jones (1952) had suggested that latter explanation for his own observations on the avoidance by some fish of reduced oxygen concentrations, but it was found not to apply to the more recent observations considered above. Hoglund (1961), who was aware of the latter results, on the basis of his own experiments also concluded that "oxygen is a non-directive stimulus to fish." While maintaining that there is no convincing published evidence that oxygen "acts as a directive stimulus," however, he offers no other satisfactory explanation of the quantitative results reported by Whitmore, Warren, and Doudoroff (1960), which indicate that even moderate oxygen deficiency, insufficient to produce acute respiratory distress, can act as such a stimulus under some experimental conditions at least. Until sufficient reasons are found for discounting the latter seemingly sound statistical evidence, the conclusion that some fish can show prompt directional changes of movement upon encountering oxygen-deficient water should not be lightly dismissed. It is noteworthy that quite different methods have been employed in the investigations that have led to different conclusions concerning the nature of the avoidance reactions observed.

INFLUENCE OF CARBON DIOXIDE

Reduced oxygen concentrations in both natural and polluted waters normally are associated with elevated concentrations of free carbon dioxide, which have been found to reduce the resistance of fish to oxygen deficiency (Alabaster, Herbert, and Hemens, 1957). In most of the experiments on the effects of reduced oxygen concentrations mentioned herein, the concentrations were reduced by means of nitrogen gas bubbled through the water, so that an increase of carbon dioxide concentration was not involved as a complicating factor. Basu (1959) found that in the presence of moderate concentrations of carbon dioxide the active oxygen uptake rates of fish are markedly reduced, especially at oxygen concentrations near the lethal levels. At any constant level of dissolved oxygen, the logarithm of the active rate of oxygen consumption usually decreased rectilinearly with the concentration of carbon dioxide. It should be noted, however, that the fish had been recently placed in the experimental media. The data of McNeil (1956) indicate that the adverse effects of moderate concentrations of free carbon dioxide on the tolerance of low oxygen concentrations by resting fish are transient, the fish evidently becoming acclimatized very soon to these carbon dioxide concentrations. Concentrations up to about 40 milligrams per liter had little effect in experiments with juvenile coho salmon when the carbon dioxide content of the water was increased to these levels and its oxygen content reduced gradually (as they usually would be under natural conditions). Also, coho salmon placed in water with a high free carbon dioxide content in sealed bottles were better able to reduce the oxygen concentration in the medium to a lower level when the initial dissolved oxygen content was high, permitting acclimation to the high carbon dioxide levels, than when the initial oxygen content was lower. Adverse effects of carbon dioxide on active oxygen consumption rates, swimming performance, etc., possibly also diminish or disappear rapidly with acclimation, so that under natural conditions the influence of carbon dioxide concentrations that are likely to occur in waters with moderately reduced oxygen concentrations may usually be negligible. In recent experiments at Oregon State University, M. L. Dahlberg found that the maximum sustained swimming speeds (mean) of largemouth bass and coho salmon at reduced dissolved oxygen levels were not affected or were only moderately affected by elevated free carbon dioxide concentrations up to 40 milligrams per liter, to which the fish had been acclimatized overnight. Concentrations near 20 milligrams per liter had almost no effect on the performance even of the salmon, which proved more susceptible than the bass to higher concentrations. Much higher free carbon dioxide concentrations do not occur ordinarily in stream waters receiving organic wastes where dissolved oxygen concentrations are not exceedingly reduced.

The reduction of the resistance of fish to low oxygen concentrations that has been observed upon addition to their medium of mineral acids (which liberate carbon dioxide from bicarbonates present in natural waters) sometimes has been ascribed erroneously to the influence of hydrogen ions. This error appears even in recent literature. For example, Bishop, (1950), in discussing the least tolerable oxygen concentration for fish as a function of pH, cites data of Wiebe et al (1934). These data, presented graphically, indicate that at reduced pH levels even as high as 6.0, bluegills are unable to extract oxygen from the water (at 18° to 25° C) when the oxygen concentration is 7 milligrams per liter or less. This cannot be true, of course, for bluegills tolerate variations of pH over a wide range. Doudoroff and Katz (1950) and Doudoroff (1957) have pointed out that the harmful effects of carbon dioxide solutions are not attributable to their reduced pH and that injurious amounts of carbon dioxide can be liberated by acids added to natural waters without reducing the pH of the water to demonstrably harmful levels. McNeil (1956) presented convincing evidence that the effects of free carbon dioxide on the dissolved oxygen requirements of coho salmon and bluegills are not ascribable to changes of pH of the water. Reduction of the pH of water to some unusually low nonlethal levels without increasing the tension of carbon dioxide (which was driven out by aeration) had little or no influence on the ability of the fish to extract oxygen from the water. Hoglund (1961) found that the avoidance reactions of fishes to increased carbon dioxide concentrations also are responses not to reduced pH, but to carbon dioxide per se.

It has long been known that fish are quite sensitive and responsive to differences of carbon dioxide concentration alone, marked avoidance reactions to elevated concentrations having been reported. Hoglund (1961), who presented additional evidence, has briefly reviewed the pertinent earlier literature. Unpublished results of experiments at Oregon State University with juvenile chinook salmon, similar to the experiments on reactions to reduced oxygen concentrations alone (Whitmore et al. 1960), indicated that, at high temperatures at least, the increase of carbon dioxide concentration that is to be expected in waters whose oxygen concentrations are reduced by natural processes could well be an important factor contributing to avoidance of such water by salmon. It is apparent, however, that the reactions of the fish to reduced oxygen concentrations per se may not yet be disregarded as being of minor importance. There is evidence that oxygen dificiency can be the chief or dominant stimulus to which fish respond when confronted with water in which the reduction of dissolved oxygen has been accompanied by a normal increase in free carbon dioxide.

PRACTICAL AND ECOLOGICAL SIGNIFICANCE OF EXPERIMENTAL RESULTS

The problems involved in the establishment of sound criteria or standards for dissolved oxygen for the adequate protection of valuable fish populations through waste disposal control have been discussed by Doudoroff (1960) and by Fry (1960). Since oxygen concentrations in waters polluted with organic wastes have been found usually to vary widely, so that average concentrations greatly exceed the minimum levels, one of the questions that has been raised (Doudoroff, 1960) is to what extent the usual fluctuations of oxygen concentrations should be considered in prescribing appropriate standards.

The Aquatic Life Advisory Committee of the Ohio River Valley Water Sanitation Commission (1955) tentatively recommended the following criteria or standards, among others, for the protection of warm-water fish populations:

> "The dissolved oxygen content of warm-water fish habitats shall be not less than 5 ppm during at least 16 hours of any 24-hour period. It may be less than 5 ppm for a period not to exceed 8 hours within any 24-hour period, but at no time shall the oxygen content be less than 3 ppm. To sustain a coarse fish population the dissolved oxygen concentration may be less than 5 ppm for a period of not more than 8 hours out of any 24-hour period, but at no time shall the concentration be below 2 ppm."

The seemingly reasonable assumption is implied that intermittent exposure of fish in their natural environment to nonlethal low oxygen concentrations during a large portion of each 24-hour day has little adverse effect on the fish if relatively high concentrations (above 5 mg/1) predominate. This implied assumption is not supported by the results of the herein reported experiments on the effects of diurnal fluctuations of dissolved oxygen on the growth of fish. Although the fish that were subjected to wide fluctuations of oxygen concentration derived some benefit from their prolonged daily exposure to high concentrations, the inhibitory influence of the recurring, intermittent exposure to low concentrations on their growth was nevertheless very pronounced. These fish did little better than those exposed continuously to the same low oxygen concentrations, and they usually did as poorly, or not as well, as they would have done at constant concentrations only about 1 milligram per liter higher than these mean daily minima. It now appears that complex and not easily enforceable water quality standards pertaining to dissolved oxygen, such as those proposed by the Aquatic Life Advisory Committee, probably are not justifiable. Simpler criteria apparently can be at least as satisfactory and defensible.

The growth rates of largemouth bass and coho salmon in the laboratory were considerably reduced even at nearly constant reduced oxygen concentrations averaging about 5 milligrams per liter and more. Although reduction of dissolved oxygen from 90 percent to 50 percent of the air-saturation value usually had a somewhat more striking effect on the growth of largemouth bass (at 25° C) than on the growth of coho salmon (at 18° to 20° C), the effect of such a reduction of dissolved oxygen on the sustained swimming speed of bass was much less pronounced than the effect on the swimming speed of coho salmon. In view of the ability of the bass to remain nearly as active at moderately reduced oxygen tensions as at higher tensions, and their relatively great tolerance of low oxygen tensions, the pronounced effect on their growth of a moderate reduction of dissolved oxygen is interesting and somewhat surprising. Can one safely conclude from the results of the growth experiments that bass production in natural situations is materially impaired at reduced oxygen concentrations near 5 milligrams per liter? Is it safe to conclude that when almost unimpaired fish production is the objective, it is at least as important to maintain dissolved oxygen concentrations near the air-saturation level in waters inhabited by the bass as to maintain such concentrations in waters inhabited by coho salmon? These questions are as yet unanswered.

The ecological significance of all the various reported observations made in the laboratory admittedly is not yet clear. Oxygen concentrations that are avoided by fish under some experimental conditions are necessarily avoided likewise under natural conditions, especially in situations where the transition from high to low concentrations is very gradual, the transitional zone extending over a long distance. Swimming speeds and developmental rates that are essential to survival and unimpaired success of fish in their natural environments, as well as larval sizes that must be attained by the time of hatching or of complete yolk absorption, are unknown. Presumably, they can all be determined experimentally, but their determination will not be a simple task.

The swimming capability of individual fish living at high oxygen concentrations in their natural environment probably can be impaired in varying degrees by some operative or mechanical means without seriously injuring them otherwise. The minimum degree of impairment of swimming performance that results in increased mortality or reduced growth rates of these fish under natural conditions presumably can be established by comparing their mortality and growth rates with those of controls placed in the same or similar natural environments. This information would indicate about how much impairment of swimming performance caused by reduction of the dissolved oxygen concentration could result in impairment of survival and growth. It must be recognized, however, that different kinds of swimming performance may not be equally or correspondingly affected by reduction of oxygen concentration. This could seriously complicate the interpretation of the experimental results.

The survival in stream-bed gravels of salmonid larvae hatched and reared at different oxygen concentrations and water velocities can be studied in the field (Coble, 1961; Phillips and Campbell, 1962) and in the laboratory. Such studies are now in progress in Oregon. There are sound reasons for believing that the relatively small and weak larvae, or sac fry, that succeed in hatching at low oxygen concentrations may be unable to survive to successful emergence from the overlying gravel under natural circumstances, even though they may do well at the same concentrations under otherwise favorable laboratory conditions. Simulation of the more rigorous natural conditions in the laboratory, where oxygen concentrations and water velocities can be accurately controlled throughout the period of embryonic development and thereafter, is deemed essential to full understanding of the fate of embryos and fry in natural redds with interstitial water of impaired quality.

The reduced oxygen concentration at which incipient impairment of the growth of fish occurs under natural conditions probably depends on the availability of food, which may itself be the major factor limiting growth rates. This "critical" concentration (for growth impairment) may well depend also on the rate at which energy must be expended by the fish in seeking and capturing the available food, while also escaping enemies, etc. The appetite of the fish, or their activity that presumably is necessary for food procurement, or both, evidently must be reduced when available oxygen becomes insufficient for supporting all metabolic processes at normal levels.

Although the growth of fish that were kept on an unrestricted diet in the laboratory where an unlimited amount of food could be found and ingested by them with minimum effort was materially impaired by moderate reduction of oxygen concentration, the growth even at very low concentrations often was quite rapid. The greatest observed growth rates of juvenile largemouth bass and coho salmon at oxygen concentrations near 2 and 3 milligrams per liter, respectively, doubtless were greater than the growth rates of these fish in many natural habitats where oxygen is abundant but the food supply is limited. When supplied only with the limited amount of food that is obtainable by fish in any one of these natural habitats, inactive fish in the laboratory perhaps would concume all of this food and grow about as rapidly at moderately low oxygen concentrations as at higher concentrations. Such a result, however, would not signify that impairment of growth of more active fish in the natural habitats would not occur upon reduction of the oxygen concentration to the same low levels. Without drastic reduction of feeding activity, the maximum possible oxygen uptake rate under these conditions could well become insufficient for digestion and assimilation of the normal food ration. Thus, the rates of food intake and growth could be reduced markedly through reduction of appetite or of success in finding and capturing food, or of both.

A satisfactory laboratory model for studies of the dissolved oxygen requirements of fish, which is directed toward reliable estimation of critical concentrations at which incipient impairment of growth would occur in a given natural environment, obviously cannot be a simple one. Evidently it must combine the food ration normally obtainable under favorable dissolved oxygen conditions in the natural environment with enforced activity approximately equivalent to the spontaneous or other activity that occurs and presumably is essential for survival and normal feeding under natural conditions. Given the mean environmental temperature, the mean daily food ration, and the normal growth rate of the fish in the natural environment, one should be able to reproduce these in the laboratory, forcing the fish to swim against a moderate current, so as to necessitate just the appropriate amount of energy expenditure through sustained activity. The minimal reduction of oxygen concentration that would result in appreciable impairment of the appetite and growth of fish in the laboratory under conditions predetermined in accordance with the proposed scheme could reasonably be assumed to correspond approximately to the minimal reduction that would impair the growth of the fish in the natural environment on which the model was based. The "critical" oxygen concentration for impairment of growth of juvenile largemouth bass, evaluated in this manner, could often turn out to be well below the air-saturation levels and below the corresponding critical concentrations for juvenile coho salmon in their typical stream habitats, where the salmon usually are much more active than bass in sluggish streams. The critical concentrations, however, at which the growth rates of a given fish species become concentration-dependent in different environments and seasons probably vary widely with temperature, the abundance and availability of food, water velocity, etc.

The growth rates of various fishes at recorded temperatures in representative natural habitats have been evaluated. Unfortunately, reliable information on the rates of food consumption by the fish and even proven methods for the evaluation of these rates under natural conditions are lacking. New methods are being developed, however (Darnell and Meierotto, 1962). Refined evaluations of food consumption rates presumably will have to be based on numerous measurements of stomach contents, together with accurate determinations of digestion rates, which are known to vary with the quality and quantity of food consumed.

The over-all research program suggested above clearly is not an easy undertaking. However, inasmuch as the suggestions apply not only to the study of dissolved oxygen requirements, but also to the study of other water quality requirements of fishes, the effort required for an effective attack on the outlined problems is deemed fully justifiable. The past research summarized in this paper is considered to be only a necessary first step toward clear definition and eventual solution of these problems.

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THE ENVIRONMENTAL REQUIREMENTS OF CENTRARCHIDS WITH SPECIAL REFERENCE TO LARGEMOUTH BASS, SMALLMOUTH BASS, AND SPOTTED BASS

George W. Bennett *

The family Centrarchidae includes many of the so-called pan fishes, as well as the larger members of the family that used to be known as black bass. As these fish are never black (with the exception of smallmouth fry), the name black bass has been discarded. This is unfortunate only because the designation "black" furnished a definite separation between the Centrarchid basses and the true basses (Serranidae).

It is safe to say that in North America there is a Centrarchid bass for every type of water warmer than that suitable for trout. These fish are found in waters with summer temperatures ranging from those of the warmest natural waters in the southern states to those of the glacial lakes along the Canadian border. Most of the species of bass are very hardy as adults, very sporting to catch, and among the most "intelligent" of fresh-water fishes. For these reasons, they have been widely distributed and are frequently found in small numbers, at least, in almost every type of aquatic habitat.

Bass are seldom caught in large numbers, although there was a substantial commercial fishery for them in the late 19th and early 20th centuries. For example, Bartlett *(1)* reported that in the year 1907, three men fishing in the Illinois River at the mouth of a slough running out from one of the backwater lakes in the Meredosia region (Morgan Co., Illinois) took 375 largemouth bass ranging in weight from one-half to 3 pounds in 3 hours. This is an average rate of about 42 bass per man-hour. This rate of catch must be considered remarkable because the average rate of catch today in heavily fished waters is probably less than one-tenth-of-a-pound per hour.

SPAWNING HABITS AND TEMPERATURES

A great deal of information has been published on the spawning habits of bass and other centrarchids, particularly those that have been raised in fish hatcheries. Nesting behavior is well known *(5)*. The male clears a circular bed, or redd, on the bottom of a lake or stream. When the nest is completed, a female ready to spawn will enter the nest and spawn with the male. The fertilized eggs sink to the bottom of the nest and become attached to gravel, rubble, sticks, roots, and other material that may be within the nest boundaries.

Kelley *(13)* reported from 5,000 to 82,000 fertilized eggs per nest for largemouth bass; Carlander *(7)* reported 2,000 to 109,000 eggs. Not all of these eggs hatched, but Lamkin *(15)* stated that he took as high as 10,000 fry from a single nest and as low as 2,000 to 3,000. Lamkin's observations were made of hatchery ponds.

Largemouth, smallmouth, and spotted bass have been reared in hatchery ponds for many years; thus a good deal of information is available on all aspects of nest building, spawning, hatching, and fry development. According to Lamkin *(15)*, when the water reached 56° F (13.3° C), largemouth bass began nest building. Actual spawning did not take place, however, until the waters were about 66° F (18.9° C).

The incubation period for largemouth bass ranges from 48 to 72 hours, depending upon the water temperature. The fry rise from the nest, that is, they resorb their yolk sacs and swim actively in 6 to 8 days after hatching. Kramer and Smith *(14)* reported that in natural waters largemouth fry rose from the nest in 5 to 8 days. These investigators recorded the percentage of nests constructed on various bottom materials: In East Slough (Minnesota) in 1958, 73 percent of the nests were constructed in the roots of water shield or water lily, 10 percent on needle rush, and the rest on fibrous debris or on sand.

The small largemouth bass reached lengths of 32.5 millimeters 26 to 31 days after hatching *(14)*. Their early diet consisted of a variety of entomostraca and small aquatic organisms. Fish appeared in the diet when the bass were 20 millimeters long.

Hatchery-reared largemouth and smallmouth fingerlings have been widely distributed in lakes and streams beyond their original range. Spotted bass are somewhat less common, but in streams where they are indigenous, they are found in intermediate temperature zones between zones in which largemouth and smallmouth bass are found. They seem to be better suited to certain types of aquatic conditions than either largemouths or smallmouths. For example, both largemouth bass and smallmouth bass have been stocked in the streams of southern Ohio for the past 40 years *(23)*. In this location spotted bass have never been stocked, yet the spotted bass is the dominant fish in these lowland streams.

The spawning temperature of the spotted bass is 64° F (17.8° C) *(10)*, which is about 2° F lower than for the largemouth bass and 2° F higher than that for the smallmouth. In many other respects the spotted bass is intermediate between the largemouth and the smallmouth; this is true even in its appearance, because it seems to have certain characteristics that are similar to those of the smallmouth and others that are similar to those of the largemouth.

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The spawning temperature of the smallmouth bass is about 62°F (16.7°C) *(22)*, or 4°F or more lower than that for the largemouth. This means that in lakes where both smallmouth and largemouth bass are present the smallmouth should spawn from 2 days to 2 weeks earlier than the largemouth.

Many observations of the effects of changing water temperatures on the developing eggs and embryos have been made by hatchery personnel. Under extreme weather conditions, temperatures in hatchery ponds may rise quite rapidly for as much as 13°or 14°F. Tester *(22)* records the death of embryos as a result of a 2-day temperature rise from 61° to 73.5°F (16.1° to 23.1°C). Drops of temperature will also cause loss of nests, partly because the parents abandon them. Lydell *(16)* reported a loss of eggs when the water temperature dropped from 65°F to 45°F (18.4° to 7.2C). Meehan *(17)* observed that the smallmouth bass abandoned their nests when water temperature dropped from 58° to 48°F (14.4° to 8.9°C). Kramer and Smith *(14)* reported the death of largemouth bass embryos when water temperature dropped from the high 60's to below 50°F.

If the male bass leaves the nest for any length of time or for any cause, death of the embryos or fry by predation usually occurs *(11)*. Nest abandonment may come about through a temperature drop, which causes the male to desert the nest. Also, when male bass are caught, leaving a nest full of fry or eggs, the nest may be lost either through predation on the fry by small sunfish, or through lack of proper aeration.

The idea of a closed fishing season during the time of spawning to protect male bass on nests has proved to be largely theoretical *(3)*. The production of new year-class of bass was less dependent upon whether or not male bass could be legally caught, than on many other factors. In the first place, bass are not stupid, and many of them refuse to become hooked. Then too, bass that are hooked or at least pricked by hooks, plugs, or bait, and escape, go back to their nest with knowledge that will prevent them from becoming hooked a second time. Murphy *(19)* studied the production of largemouth bass fry in parts of a lake in California that were closed to fishing and in other parts that were open to fishing during the spawning season; he concluded that there were no more small bass in areas that were closed to fishing than in areas that were open. Later, during a year when the entire lake was open to fishing, there were more small bass available than were present in any situation in the year of the original study. There is overwhelming evidence, however, that the vulnerability of the eggs, fry, and the young during the early months of life control the size of the bass populations.

Kramer and Smith *(14)* stated that year-class strength of largemouth bass was set during the first few days or months of life and was affected by a number of variable factors, including the size and fecundity of spawning stock, weather, physical-chemical and biological conditions of the habitat, food, and predation. Bennett *(3)* could demonstrate no relationship between number of spawners and the size of a year-class produced.

SENSITIVITY TO CHANGE IN pH AND DISSOLVED OXYGEN

Bass are not very sensitive to moderate changes in their environment as long as these changes do not exceed a certain definite range. For example, largemouth bass fingerlings, 3.5 to 6.0 inches long, tolerated rapid changes in pH from 8.1 to 6, from 7.25 to 9.3, from 9.2 to 6.1, and from 6.1 to 9.5 *(24)*. In other experiments, Wiebe determined that bass were not affected by excessively high dissolved oxygen.

Moss and Scott *(18)* made a series of experiments in which they tested the minimum oxygen requirements of small largemouths. Using a shock treatment technique in which the dissolved oxygen was rapidly reduced from near saturation, they found that at 25°C the minimum requirement was 0.92 ppm. At 30° C the minimum was 1.19 ppm, and at 35°C the minimum requirement was 1.40 ppm. When these bass were acclimated gradually to low oxygen they were able to get along on somewhat smaller amounts. For example, at 25°C the requirement range of acclimated bass was 0.78 to 0.84 ppm; at 30°C from 0.79 to 0.87 ppm; and at 35°C, 1.20 to 1.32 ppm. It is probable that spotted bass and smallmouth bass would die at higher minimum oxygen levels than the largemouth bass.

LETHAL TEMPERATURES

Brett *(6)* lists upper and lower lethal temperatures for largemouth bass that previously have been acclimated to temperatures of 20,° 25,°and 30° C. These are shown in Table 1.

Table 1. LETHAL TEMPERATURES OF LARGEMOUTH BASS

High temperatures		
Acc. Temp.		Lethal Temp.
20° C (68° F)	—	32.5° C (90.5° F)
25° C (77° F)	—	34.5° C (94.1° F)
30° C (86° F)	—	36.4° C (97.5° F)
Low Temperatures		
Acc. Temp.		Lethal Temp.
20° C (68° F)	—	5.5° C (41.9° F)
30° C (86° F)	—	11.8° C (53.2°F)

Similar tests made on bluegills indicated that they were more sensitive to high temperatures than largemouths but somewhat superior in their ability to withstand low temperatures. Similar information for spotted and smallmouth bass is apparently not available.

PREFERRED TEMPERATURES

According to Fry *(9)* fish prefer a certain temperature range which is "the region, in an infinite range of temperature, at which a given population will congregate with more or less precision -- a temperature around which all individuals will ultimately congregate, regardless of their thermal experience before being placed in the gradient." Ferguson *(8)* lists field observations of the preferred temperature for largemouth bass as 26.6° to 27.7°C (79.9° to 81.9°F), spotted bass as 23.5° to 24.4°C (74.3° to 75.9°F), and smallmouth bass as 20.3° to 21.3° C (68.5° to 70.3°F). The preferred temperatures for largemouth and spotted bass were based, however, on field observations in Tennessee and the smallmouth from northern Wisconsin. One might assume that smallmouths from northern Wisconsin would demonstrate a lower preferred temperature than this species in waters farther south where summer water temperatures normally greatly exceeded 21°C (69.8° F). Laboratory tests with smallmouths showed the preferred temperature was 28°C (82.4° F), but such tests with both largemouths and smallmouths showed higher preferred temperatures than those recorded in field observations.

THE EFFECTS OF COMPETITION WITH OTHER SPECIES

Individual species of fish inhabiting an aquatic habitat are affected by other species of fish inhabiting the same water. These other species may serve as food for piscivorous species if the former are of such sizes as to be edible. If they are too large to serve as food, it is probable that their offspring serve such a purpose until they, in turn, grow too large. As suggested by Kawamoto *(12)*, Rose *(20)*, Swingle *(21)*, and Yashouv *(25)*, fishes may influence one another by the secretion or excretion of waste products or hormonal substances that may inhibit growth or reproduction of individuals of the same species or of other species inhabiting the same water. This is competition for space, a phenomenon that has been recognized, but until recently very little has been done about it. Yashouv *(25)* demonstrated that fishes were able to excrete a substance, which, when added to an aquarium containing other fishes, prevented these other fishes from making normal growth. When this substance was given to rates in their drinking water the result was similar to that of vitamin B_1 deficiency, and eventually resulted in the death of the rats.

Swingle *(21)* found that goldfish held in relatively large numbers in small ponds would not spawn regardless of the season. As soon as a few of these fish were removed from the concentrated population and placed in a pond containing fresh water, they ripened and usually spawned within 48 hours. Carp, buffalo, and some other species are assumed to show similar repression as a result of substances secreted or excreted into the water. So far, there is no evidence that any of the basses or centrarchids are inhibited in this manner. Certainly overpopulations of stunted bass, and/or sunfish, including bluegills, green sunfish, and others are relatively common. There is no evidence that their reproduction is inhibited, except through predation on eggs and newly hatched fish that normally takes place in concentrations of this kind.

This inter-predation of bass on sunfish and sunfish on bass spawn is a very potent factor in the ecology of all of the bass species. Over a period of years evidence has accumulated that the number of largemouth bass, smallmouth bass, and probably spotted bass as well, is often controlled by the species of fish that inhabit the same water with them *(2,4)*. Actually, when a new body of water is stocked so that it may develop a dominant brood of largemouth or smallmouth bass, these fish temporarily control other species stocked with them; i.e., the young spawned by the bluegills in a bass-bluegill population may be controlled by the bass for the first year or two, provided the number of bass stocked originally is sufficient. Control is not complete, however, and these sunfish species manage to produce additional young, with sufficient survival to allow the gradual and slow build-up of a population of sunfish. Once this population of sunfish approaches a density of 4 to 5 thousand individual fish per acre, of sizes between 4.5 and 5.5 inches, they offer such severe food competition (in most aquatic habitats) to one another that they are constantly ravenous and attack any source of food available. Bass guarding nests of eggs or newly hatched fry are surrounded by milling schools of hungry sunfish ready to make inroads on these eggs and fry if the opportunity arises. Such schools of predatory sunfish eventually stimulate the guarding male bass to chase and strike a fish that comes too near the nest. While this guarding male bass is gone momentarily, sunfish nearest the nest dive in and eat eggs or fry as rapidly as possible. When the male bass returns these fish scatter, but in the short interim when he was gone they consumed perhaps as many as 100 eggs. One can see that when this predation continues over the days to hatching, yolk absorption, and schooling, the usual end result is that the nest is a total loss. Thus, inter-specific predation on the developing bass, allows sunfish to control the total survival of these bass. Of the three species of bass considered here, probably the largemouth is most capable of protecting its young.

Populations of fish are common that consist of stunted bluegills, or crappies, or green sunfish, or a combination of two or more of these species, and a few large, well-fed, old bass. These bass are unable to produce a new year-class because of the inroads made upon their reproductive products at the time of nesting. A part of the reason that the largemouth bass is less vulnerable to this type of attack than is the smallmouth is that the largemouth young not only are very active, but also they swim in a compact school which is more easily guarded than is the scattered school of the young of the smallmouth bass. One compensatory factor may be the fact that the newly hatched smallmouth fry are similar in coloration and size to toad and frog tadpoles, which appear in the spring at about the same time. These tadpoles are not considered very desirable as food, and for this reason they are at least partly immune from attack. Field observations indicate that smallmouth fry are protected to a certain extent during the very early free swimming stages by these similarities to tadpoles. When the smallmouths reach lengths of about 20 to 25 mm, however, they become light in color and take on the markings of the adult fish. At this time, they are much more active than when they first leave the nest, and become scattered in shallow water.

One reason why smallmouth bass are unsuccessful in typical warm-water ponds is because they apparently offer poor competition to the normal population complex of these warm-water ponds *(4)*. I am referring to such fish as green sunfish, bluegills, bullheads, and orange-spotted sunfish, which are normally found in and often dominate warm-water pond habitats. If breeding smallmouth bass are introduced into a pond populated by these warm-water species, the former may spawn, but the survival of their young may be nil or very low. In a number of experiments smallmouth bass were found to do well and reproduce successfully in warm-water ponds as long as these sunfish were present in small numbers, or as long as the bass were stocked with those species with only little tendency to overpopulate and stunt. For example, 25 smallmouth spawners were used to stock a 14-acre warm-water pond. After the first spawn, the pond contained a substantial population of smallmouth bass that was maintained through natural reproduction for several years or until green sunfish expanded to a population of several thousand fish per acre. At that time, the population of smallmouth bass was very much reduced and there was no evidence of their ability to reproduce while living with an overabundance of green sunfish. When smallmouths were exchanged for largemouths in the same lake and subjected to essentially the same type of competition, the largemouth bass were not so readily controlled as were the smallmouths.

DISCUSSION

The information presented here does not define the environmental requirements of bass and other centrarchids; it has been presented to show that these requirements are not very specific, and that vulnerability of bass is restricted largely to early stages in the life cycle, from embryos in the nests to advanced fingerlings. Probably this high vulnerability of young is common to most animals.

Factors other than undesirable water characteristics may decimate bass populations in aquatic situations dominated by man. This is because the basses thrive under situations where natural fish predators are abundant and not where overpopulation and stunting of companion species is the rule rather than the exception. Where man dominates an aquatic environment, fish predators are scarce or nearly absent. Bass are often scarce, not because the water is polluted (as it may well be) but because the biological aspects of the aquatic habitat are less than optimum for them.

Thus, the absence or scarcity of bass in a body of water is not an indication that the habitat is chemically or physically unsuitable for their survival.

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WATER QUALITY CRITERIA FOR FISH LIFE

Marcel Huet *

INTRODUCTION

Surface waters are not all identical. Different classifications can be established:

1. Those waters that provide drinking water for the country.
2. Waters used for agricultural purposes and for fish culture.
3. Waters whose main use is to fulfill industrial needs.
4. Waters used for recreation and water sports.

It is not easy to define the qualities of water beneficial to fish, and in any case, it cannot be achieved by merely listing numerous chemical-physical values.

Undoubtedly it is more difficult to define the qualities of water beneficial to fish than the qualities essential for drinking, industrial, or agricultural waters. Indeed, this difficulty has been stressed by the "Aquatic Life Advisory Committee Commission" (1955). This Committee points out that water cannot be considered pure from a piscicultural point of view if, after pollution, it does not produce the quantity of fish it should. Neither will it be considered pure if, after several changes and especially after a constant rise in temperature, it produces fish of less economic or sport value. Futhermore, water is not regarded as pure if fishing is affected or if the quality of the fish flesh is altered while the quantitative production is not affected (Huet, 1949).

In the first part of this paper we shall consider in general the main qualities of water necessary for fish; in the second part, we shall enlarge on certain essential chemical-physical characteristics.

I. PRINCIPAL QUALITIES OF WATER FOR FISH.

A. Essential needs of fish.

In order that a given population of fish can exist, it is necessary that the fish be able to live and develop normally. This means that the ecological conditions must be satisfactory.

1. The respiratory needs of fish.

The needs are very different from one species to another. Certain species such as salmonids, require high dissolved oxygen (DO) concentration, while other kinds, such as many fish from tropical regions, only require lower concentrations. If, for western temperate Europe, one takes the required DO concentration of the tench as standard, that of the carp is two and a half times greater and those of the rainbow trout six times greater.

2. Food requirements of fish.

All fish do not have the same feeding habits. It is possible to differentiate immediately between the feeding habits of young and adult fish. In their early stage, all fish feed on microorganisms; later, their feeding habits differ. The main divisions are as follows:

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(1) Fish eating aquatic plants (algae or higher plants).

(2) Zooplankton feeders among which the following distinctions must be made: (a) Those feeding on bottom fauna. (b) Those feeding on fauna living among the submerged vegetation. (c) Those feeding on plankton.

(3) Mainly or exclusively piscivorous fishes.

All types of water do not have the same nutrient content. Waters range from those with high nutrient levels to ones practically sterile. The relative nutrient level of a water is difficult to measure. One method is to determine the "biogenic capacity" (Huet, 1949) of the water.

Whatever the feeding habits of fish in a specified situation, their food corresponds to one or several phases of the biochemical cycle that exists in the environment. This cycle originates from the nutrients in solution in the water.

3. Reproductive requirements.

The most important reproductive requirements are temperature and a suitable substratum. Details on the importance of temperature on fish reproduction are given later. A suitable temperature is necessary for warm water species as well as for cold water forms.

The type of substratum is extremely important. Many fish lay eggs that stick to water plants, and the presence of these plants is essential for the reproduction of these species. In artificial lakes subject to considerable fluctuations in water level and situated at low or average altitudes, the water heats sufficiently in the spring to allow the cyprinids and accompanying predators to reproduce. These fluctuations prevent the growth of aquatic vegetation and make reproduction of these species virtually impossible. In modern canals and in rectified streams with stone or concrete embankments, absence of vegetation also occurs.

The nature of the stream bottom also plays an important part. Trout kept in ponds or even in fresh water streams with a sandy bottom do not reproduce. They require fast flowing streams with stony or gravelly bottoms; in these conditions, they can lay their eggs and the stream flow provides a constant supply of oxygen-rich water.

Spawning sites are essential for fish reproduction; equally important is the free access to these spawning places. Access can be inhibited or made impossible by dams and pollution. The disappearance of salmon from many streams is a well-known example. It should be added that damming often warms or cools the water. This in turn can cause a modification in the composition of the fish population downstream from the dam. It can also cause an overcrowding of the water upstream by those species which are regarded as undesirable and which are increasing in the impoundment.

4. Conditions of habitat.

Certain species of fish live in free water, either in the median zone of streams or in the pelagic region of lakes and ponds as for example, the Coregones that do not have any other special habitat needs. Other types of fish living in the marginal or littoral zones of streams need aquatic vegetation and the protection of the roots and shadowy recesses of the riverside trees. Trout, for example, need sheltered spots and hiding places. If this protection is missing as a result of damming or stream improvements, the fish leave the unsuitable habitat.

In order for a species of fish to remain in a particular spot, it is essential that all the needs mentioned above be satisfied. Cases exist where the food requirements are satisfied, but the DO requirements are not met.

B. Classification of continental waters.

All continental waters, therefore, should satisfy the fish's relative needs for dissolved oxygen, food, and special conditions for reproduction and habitat. But these needs themselves are very different depending on the kind of water one is considering. By considering only the broad lines of a generalized fish classification, natural waters and artificial waters can be distinguished; the first group can be subdivided into streams and standing water.

Beforehand or concurrently, another distinction should be established concerning climatic zones and the difference between continental waters of temperate zones and those of tropical regions. Here, only the waters of temperate regions are reviewed.

Streams form a network of communication throughout a continent, linking the mountaintops with the ocean; they are fed by the melt waters of glaciers, by surface runoff, and from springs. Streams exhibit many aspects and are inhabited by many different species of fish, according to the fish zone that exists.

A stream flowing down from the mountain top to the sea includes a series of zones, each of which has a characteristic species of fish that can best thrive there. From headwaters to mouth, one observes successively, the trout zone, grayling zone, barbel zone, and bream zone. At the mouth of the rivers the sparling zone also occurs, influenced by salt water through the action of tides. This classification is essentially the result of a joint action of temperature and water velocity. The trout zone and grayling zone form the salmonid region; the upper type of barbel zone forms the mixed streams (fish population with a predominance of running water cyprinids and of salmonids), and the lower type of the barbel zone and the bream zone form the cyprinid regions. Details on the fish populations of these zones, and the method of identifying them by examining their length profile and their slope have been given in one of our articles to which we refer (Huet, 1954).

Among the natural standing waters are lakes, ponds, swamps, and marshes. This classification is based on the size and depth of these waters. Numerous bases have been suggested for the classification of lakes. From the piscicultural point of view, for temperate lakes one can adopt a similar classification to that adopted for streams. Thus, one can distinguish between salmonid lakes, mixed lakes, and cyprinid lakes. Similarly, this classification is also based on the temperature of the water and the amount of oxygen in the water. Fish populations of various categories of lakes are similar to those in the corresponding categories of streams. Important differences occur, however, because of the existence in the lakes of a pelagic region, which contains a particular population.

Artificial waters include ponds and lakes. Artificial lakes can be classified on the same basis as natural standing water. The least satisfactory for the fish are those subject to severe fluctuations in water level. The reasons for this have already been pointed out.

Artificial ponds are devoted to fish-rearing (fish-culture) or to sport fishing. Basically, one differentiates between cyprinid ponds and salmonid ponds which need, respectively, warm water or cold water in the summer. The summer temperature of 20°C marks the separation of these two kinds of fish culture.

The cyprinid-culture is concerned with the breeding of carp, tench, other cyprinids and their corresponding predators, pike, and black bass. These species are reared in shallow ponds, at low altitudes with only a weak current or none at all. In salmonid culture, many kinds of fish are reared, especially the common trout and rainbow trout. They are reared in waters that remain cool in summer and which are, consequently, found in mountainous places or, if on plains, next to large springs.

II. ESSENTIAL CHEMICAL-PHYSICAL CHARACTERISTICS OF FISH WATERS

In this second part a few details on points arising from certain essential chemical-physical characteristics of fish waters are given. Although it is difficult to make a firm choice of these properties, one can split them into two groups: those essential to the life of the fish and to the organisms that affect this life, and those which, although necessary, affect mostly the intensity of production.

Among the first group the following must be listed: temperature, amount of dissolved oxygen, pH, suspended matter, dissolved toxic materials, and properties that give an unpleasant taste to the flesh of the fish. In the second group are nutrients dissolved in the water or contained in the upper layers of the bottom of the ponds; in optimum proportion, these enhance fish production, especially primary production. Deficiencies of nutrients reduce the production. These nutrients are salts of calcium, phosphorus, potassium, nitrogen, iron, magnesium, and other elements such as copper, cobalt, and zinc. The abundance of these mineral elements determines the water productivity. A study of this aspect of the problem is beyond the scope of this present treatise. Several writers, notably Schaperclaus (1961), have contributed to our knowledge on this subject and have enlarged upon the role of nutrients in fish production.

Pinpointing criteria for fish is particularly complex and difficult and cannot be done without a wide knowledge of the needs of fish, with regard to the water milieu. That is the reason why these needs were discussed in the first part of this paper. From the points which follow, it can be seen that in general one can make only relatively precise, clear-cut criteria on temperature, dissolved oxygen, and pH.

Care must be taken to avoid confusing the resistance limits of these factors with the optimum values admissible for the same factor, that will safeguard the life and normal development of the fish.

One discovers numerous references about the effects of substances or factors that are lethal to fish after a fairly short time. Such facts are interesting for establishing those conditions that are lethal to fish; but, above all, what should be defined are those conditions that are indispensable for the life of the fish and its normal activities.

A. The temperature.

The water temperature is a critical factor in the life of fish and other water organisms and consequently in fish production. It affects to a considerable degree respiration, growth, and reproduction of fish.

Each species of fish has a thermal tolerance zone in which it behaves in a normal manner; also, there is a zone of higher temperature and one of a lower temperature in which the species can survive for a certain length of time. A gradual and regular acclimatization allows certain species of fish to survive in temperatures that would be fatal if they occurred suddenly. Fish adapt themselves quickly to a rise in temperature, but less easily to a drop in temperature.

The Aquatic Life Advisory Committee of the Ohio River Valley Water Sanitation Commission (1956) points out quite rightly that a rise in temperature of 5°C can completely transform a water milieu. The Rivers Pollution Prevention Sub-Committee of England (1949) points out on the other hand that during the summer period, a rise in temperature of 1°C can have adverse consequences.

1. Water Temperature and Fish Respiration

Water temperature determines, to a large degree, the amount of dissolved oxygen in the water, and this is essential for the breathing of fish. The needs of fish differ widely according to species. Water temperature ought not exceed: 20°C in salmonid waters; 22°C in mixed waters; and 25°C in cyprinid waters.

An increase of 1°C or even 1.5°C can temporally be tolerated for salmonid and mixed waters. For cyprinid waters, this tolerance can be extended to 2°C or even 3°C.

As a whole, fish of temperate regions are able to tolerate temperatures ranging between 0° and 30° C; but the resistance of the various types of fish to the highest temperatures in this thermal zone differs from one species to another. The above-mentioned cold water fish (salmonids, for example), do not tolerate high temperatures while the warm water fish (cyprinids in general and particularly carp) tolerate high temperatures perfectly well and, to a certain degree, even require them. In temperate regions, fish tolerate temperatures of less than 4° C. This does not apply in tropical regions; for example, most *Tilapia* sp. die when the temperature drops below 13° C. In general, all abrupt variation in temperature can be harmful to fish, even if it is short lived. Nevertheless, the dangers of sudden variations have been exaggerated in the past.

Predators and cyprinids are more sensitive than salmonids. In the case of a low concentration of oxygen, the sensitivity is increased. Whatever the species, it is prudent to avoid sudden and abrupt variations of temperature if the temperature variation of the water falls below 6° C. Several writers recommend avoidance of sudden variations in temperature exceeding 5° C. There are different opinions about the tolerance of fish to prolonged or temporary rises of temperature. For example, Tarzwell (1957) mentions that brown trout and rainbow trout have been capable of withstanding temperatures as high as 28.3°C. Black (1953), however, states that 24°C. is a lethal temperature for rainbow trout fingerlings. This limit seems relatively low. Denzer (1952) fixes the extreme of this danger zone at 24.5° C for rainbow trout fingerlings, and the ideal temperature for this same type at between 9°C and 18.5 C Fry (1951) states that brook trout fry have tolerated temperatures of 23.5° C for 12 to 14 hours. The lethal temperature for *Salvelinus namaycush* is 23.5 ° C and the best temperature for this type is between 15°C and 17°C (Gibson and Fry, 1954). Research on several salmonids, undertaken by the Southern Research Station, Maple, Canada, shows that the lethal temperatures of various salmons, *Critivomer namaycush* and *Salmo fario* is between 23.5° C and 25.3° C, depending on the types and experimental conditions.

According to Liebmann (1960), pike can tolerate temperatures up to 29° C.; most of the cyprinids can survive up to 29°C to 31°C and even higher; carp can go as high as 36°C to 37°C and carassins can even resist temperatures up to 39°C.

The preceding values are a little higher than those quoted by the Rivers Pollution Prevention Sub-Committee of England (1949), whose lethal limits are 25°C for common trout; 30°C for pike; and 35°C for the goldfish. This Committee suggests that these same types of fish can live during prolonged periods at temperatures to 22°C for trout; 27°C for pike; and 30° C for carassins, as long as the transition to the higher temperatures is carried out slowly and progressively. Abrupt changes of temperature are regarded as harmful or deadly to the fish. In the second Progress Report of the Aquatic Life Advisory Committee of the Ohio River Valley Water Sanitation Commission (1956) can be found the lethal temperatures for many kinds of fresh water fish of North America.

2. Water temperature and the growth of fish.

Water temperature influences the growth of fish considerably. Growth of carp is very good between 28°C and 20°C average between 20°C and 13° C, poor between 13° C and 5°C, and non-existent below 5° C. The optimum temperature varies according to type. In general, the best temperatures for growth of salmonids lie between 15°C, and 18° C. It is desirable that in water where one is raising one or more species of fish there should be a temperature close to the optimum of the fish in question for as long as possible.

3. Water temperature and fish reproduction.

Temperature is a factor in fish reproduction in a special and important manner. Fish only spawn when the water reaches a suitable temperature. In western Europe, some fish spawn in winter at low temperatures; e.g., salmonids. Pike spawn in the spring with the first warming of the water when the temperature reaches 10°C; the common perch spawn a little later when the water temperature reaches or exceeds 12°C; most of the cyprinids spawn at the end of spring in water temperatures of 15°C, and above. The chevinne need 12°C to 15°C, the roach 12° C to 16° C, and bream need 17° C. For carp the water temperature must be 18° C, and higher for tench.

It is essential that the required temperature should be reached at the right time; if not, the fish do not breed. Cyprinids, for example, do not reproduce if the water is not sufficiently warm in the spring; on the other hand, salmonids do not reproduce if the water does not get cold enough in the autumn. This factor limits the reproduction of trout in tropical regions. According to the Rivers Pollution Prevention Sub-Committee of England (1949), the eggs of the common trout cannot withstand a temperature higher than 14.4°C.

Any abnormal variations in temperature during the period of reproduction, causing either a sudden raising or lowering of the water temperature, are especially injurious. An early heating of the water in spring can initiate reproduction of the warm water fish of temperate regions but the eggs or the fry are destroyed by a subsequent cold spell.

B. Disolved oxygen.

The importance of this element, essential for the respiration of fish, is very marked. Fish are very sensitive to any decrease in the volume of oxygen and die of asphyxia very quickly. It is important, therefore, that fish never have to withstand the lethal dose even for a short period.

The amount of dissolved oxygen in the water depends mainly on the water temperature, the amount of organic matter, and underwater vegetation. In natural waters, the most important factor influencing the amount of dissolved oxygen is temperature. Temperature is important because the more the temperature rises, the less dissolved oxygen there is and the oxygen consumption especially through the oxidation of organic matter is increased.

The amount of dissolved oxygen also depends on the quantity of organic substances found in the water. If this quantity is high, the oxidation of these substances uses up a considerable amount of the dissolved oxygen in the water, and the concentration can decrease below the necessary minimum. This can happen from natural causes. It happens during the stagnation period in the hypolimnion of eutrophic lakes; it also happens in winter under the ice in stagnant water that has a weak current; it happens often in water polluted by organic matter, from domestic sewage, and some types of industries.

The amount of dissolved oxygen normally required by fish and the minimum concentration they can tolerate differ drastically from one type to another. One finds many facts and opinions on this subject and the resultant figures have little similarity. This is not surprising if one considers the variety of types of fish studied, the conditions of the study, whether in natural water or in the laboratory, the methods of investigation, and the interpretation of the results (Tarzwell, 1957).

The average minimum dose permissible for carp is from 3 to 3.5 milligrams per liter, and for the trout from 5 to 5.5 milligrams per liter (Schaperclaus, 1954). Quantities that are markedly lower can be tolerated but only for a short time. They can drop to 1.5 or even 1.0 milligrams per liter for trout and 0.5 milligrams per liter for carp and tench. There are no absolute minimum amounts supported by fish, they depend on many ambient factors mentioned above, most notably the temperature, and those properties of the fish itself, such as species, age, size, and activity. Young fish or breeding fish have greater needs. Salmonid eggs lying in the gravel need water rich in oxygen.

In practice, normal natural waters must subscribe to the following conditions. Within the limits which have just been laid down, salmonids and mixed waters should have a dissolved oxygen concentration of at least 80 percent of saturation; locally and temporarily, this amount can be reduced to not less than 5 milligrams per liter. In cyprinid waters, the amount of dissolved oxygen should normally be at least equal to 70 percent of saturation: locally and temporarily the amount can fall to 3 milligrams per liter.

For water equivalent to European cyprinid waters, Tarzwell (1957, 1958) recommends that the oxygen concentration not go below 5 milligrams per liter for more than 8 hours in 24, and never below 3 milligrams per liter unless the population is composed only of coarse fish and not accompanied by predators having greater oxygen demands. If there are only coarse fish, the amount can go as low as 2 milligrams per liter. For salmonid waters Tarzwell recommends a minimum of 6 milligrams per liter. Others (Discussion by Perry of Tarzwell, 1958) estimate 6 milligrams per liter as insufficient and suggest probably quite correctly 7 milligrams per liter as a minumum amount for salmonid waters. As mentioned above, minimum values of 5 milligrams per liter for salmonid waters and 3 milligrams per liter for cyprinid waters have been given by other writers (Ellis, etc., 1948). Graham (1949) has determined that the activity of brook trout is reduced below 75 percent saturation whatever the temperature, and suggests that above 20° C saturation is needed to allow normal activity.

Fry (1951) estimates that an oxygen concentration of at least 6 milligrams per liter is needed for normal development of salmon eggs and fry. For normal activity of brook trout, he estimates 7.6 milligrams per liter at 15° C, and saturation if the temperature is 2° C or more.

One should fix standards for the concentration of dissolved oxygen in water; it is also necessary, however, to fix the biochemical oxygen demand (BOD) for a period such as 48 hours. The BOD after 48 hours should not normally exceed 25 percent of the original amount.

C. pH.

The best water for fish is that which possesses a slight alkaline reaction -- a pH between 7.0 and 8.0. Fish can tolerate acid or alkaline pH levels but the limits should be between 5.0 and 9.5.

Temporarily lower or higher values can be reasonably well tolerated for various periods depending on the type of fish. Tarzwell (1957) points out that certain fish have been able to live for long periods at pH of 4.5 to 4.2. The extreme limits that fish cannot withstand are close to 4.0 and 10.0. One can find details concerning the pH values that have been tolerated by various kinds of fish. All species of fish do not resist equally a low or high pH. Carp are fairly sensitive to a low pH; such fish do not tolerate a pH less than 5.0 for long. Trout eggs die in a pH of 4.8.

The pH of the majority of natural waters lies between 6.0 and 8.5, but in moor water it can drop to 4.4. Wherever possible, it is desirable to maintain the pH between 6.5 and 8.5. Sudden and appreciable variations of pH in the water are undesirable.

It is important to point out that pH has an important indirect effect because of its influence on the toxicity of certain substances, such as HCN, H_2S, and NH_3.

D. Suspended matter.

A sufficient but not excessive limpidity is a property of good water for fish. The limpidity must be sufficient because turbidity acts in an unfavorable way on the productivity of the water. The shortage of light prevents or interferes with plankton, biological covers, and aquatic plants, resulting in a lower nutritional value of the milieu. Tarzwell (1957) mentions that one finds seven times fewer organisms in a river downstream from a mine than upstream.

The turbidity of water can be due to natural causes such as milky and cold water caused by melting glaciers or heavy rain, melting snow, debris, and dead leaves. But turbidity can also be the result of pollution caused by quarries, cement-works, gravel-pits, and even by numerous industries manufacturing organic materials. Turbidity can be caused by solid particles that settle quickly or by colloidal matter that remains in suspension for a long time. Erosion from various causes, especially deforestation, can have serious and damaging consequences. This is an example of what is happening in the United States.

Water turbidity can be harmful to fish not only indirectly by reducing the nutritive value of the milieu, but also directly if the suspended particles harm or block the gills. It must be pointed out, however, that fish easily tolerate high turbidity for long periods except when the turbidity is accompanied by acids, allkalies, or substances that injure the gills and prevent their functioning normally.

The resistance of fish to turbidity varies according to type. Carp and silurids tolerate a high degree of turbidity. This does not apply to predators and is perhaps the reason for the inopportune propagation of the carp in certain parts of the United States (Huet, 1959).

In general, turbidity is more harmful to the nutritive richness of the water than directly to the fish. Disturbed water and the resultant deposits can also interfere with or completely prevent the reproduction of fish by destroying the eggs or the breeding places.

High transparency is also an indication of nutritive deficiency. The difference between oligotrophic lakes and eutrophic lakes is well known. In the former transparency can reach 25 meters or more, while in the latter where water is productive, it can fall to less than 0.50 meters as a result of considerable plankton development.

The Aquatic Life Advisory Committee of the Ohio River Valley Water Sanitation Commission (1956) estimates that in order to maintain the most favorable conditions for the growth of the more important water plants, it is essential that the water transparency should be such that at least 25 percent of the sun's total radiation reaches the plants.

E. Dissolved matter.

Pure water for fish must contain sufficient quantities of nutritive salts necessary for plant and animal life in the water. These salts exist in innumerable quantities and new ones are made daily. Substances exist in certain natural waters that are harmful to the fish such as excess iron, sulphur, salt water, or moor water. Most of the substances toxic to fish are put into the water by man. Several writers, among them Steinmann (1928), Ellis (1937), Doudoroff and Katz (1950 and 1953), Vivier (1957), and Liebmann (1960), have contributed to the literature on this subject.

Fish toxicology is a very complex science. For each substance one attempts to determine several values:

(1) The "minimum lethal dose" (Todlich keits- oder Letalitatsgrenze), which is reached when the concentration employed still causes the fish's death during the standard experimental period.

(2) The "dilution limit" (Empfindlichkeitsgrenze), which is reached when the concentration employed does not cause unfavorable effects on the fish, either during the standard experimental period, or during the course of the next 2 days.

In reality, the study of the toxicological phenomena is much more complex. Wuhrmann and Woker (1948) distinguish between a latent phase and a lethal phase, each of which has its own important characteristics.

Several writers, notably Doudoroff (1951), conducted experiments to discover the "Median Tolerance Limit (TL_m)" which is the concentration at which a substance or an effluent kills 50 percent of the fish during the period of the experiment (48 hours, for example). The permissible tolerance for waste waters is obviously less and as a rough approximation can be fixed at only 0.1 of the TL_m; this value of 0.1 is clearly subject to verification and modification, however.

Facts arising from research on fish toxicology are difficult to establish and interpret, unfortunately. Numerous specific and ecological factors determine variations in the results of toxicology tests. Among the specific factors are the type of fish (there are considerable differences in the sensitivity of different types -- trout are very sensitive, the pike less so, and carp and tench considerably less); the age of fish (young fish are often more sensitive than adults); the physiological state (breeding period); and the state of health. Important among the ecological factors are temperature (a rise in temperature usually aggravates the toxicity); the amount of dissolved oxygen (the toxicity of many substances increases if the amount of dissolved oxygen in a particular place is reduced); and the chemical composition of the water.

Variation in the effects of toxic substances is produced by differences in the composition of the receiving waters. Therefore, it is of little practical use to refer to the literature about the toxicity of materials in complex water containing different substances. The results of many past experiments are of little use because the composition of the water in which they were conducted is not known. Another complication arises from the fact that discharged substances are mixtures whose reactions are different from their components. Synergistic or antagonistic actions often result.

On the question of the toxicity of complex waste water, the best method of approaching the problem is to do the experimental tests with the whole effluent and dilute with water from the receiving stream collected at the place where the waste is being discharged (Tarzwell, 1957).

The influence of temperature and the amount of dissolved oxygen on the toxic effects of fish poisons has been particularly stressed by Wuhrmann and Woker (1955).

The influence of pH on the toxicity of several substances has been mentioned above. For many substances (weak inorganic acids and organic acids) a decreased pH increases the toxicity. Ammonia becomes much more toxic if the pH increases above 8.0. The influence of pH on cyanide is particularly great. Doudoroff (1956) discovered that the nickel cyanide complex was tolerated a thousand times better by the fish at a pH of 8.0 than at 6.5.

With a view to coordinating the toxicity tests and making their results comparable, a standardization of tests was proposed by Swiss, French, and Belgian experts at a meeting in Zurich, in 1952. The length of the experiment is obviously of great importance; for practical reasons, one stops often after a period of 6 hours. Doudoroff et al. (1951), Herbert (1952), and Henderson and Tarzwell (1957) have suggested methods for conducting toxicology tests.

F. Substances giving a bad taste to the flesh of fish.

Water for fish production should be free from substances capable of giving a bad taste to the flesh of the fish. Certain substances, especially phenols, are toxic to fish at a certain concentration. At greater dilutions, they no longer threaten the life of the fish, nor are they harmful to the fish food organism, but they give such an obvious and unpleasant taste to the flesh of the fish that they are rendered unpalatable. Because of this, fishing as a sport and for commercial purposes is seriously impaired.

The unpleasant taste of the fish flesh can come from natural causes as for example the muddy taste given by the blue-green algae of the *Oscillatoria* species. In fish culture the taste of the flesh can be influenced by bad food. Often in flowing water, the taste of fish flesh is altered by waters that influence the fish directly or through the contaminated food they eat. Petrol, mineral oils, water from refineries, chlorophenols, and phenols are especially offensive. Phenol concentrations of 0.1 milligram per liter, and even as low as 0.02 to 0.03 milligram per liter are enough to contaminate the flesh of the fish, particularly if they are kept in boxes. Several weeks are needed to rid the flesh of a fish of the unpleasant taste it has acquired.

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TOXICITY OF SOME HERBICIDES, INSECTICIDES, AND INDUSTRIAL WASTES

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Results of studies conducted in the laboratories at the Station of Applied Hydrobiology on the toxicity of different compounds are presented here.

Some tests were continued over a period of 1 or 2 months while others were limited to 6 hours as specified by the "protocole de Zurich" adopted between Belgium, Switzerland, and France.

BIOASSAYS CONDUCTED WITH HERBICIDES

1. Simazine (2-chloro-4-6 bis-Ethylamino triazine) is employed in the commercial product "Herboxy" at a concentration of 50 percent. Long-term tests were conducted in small aquaria where reaeration was obtained by means of a small pump. The fish chosen was the minnow *(Phoxinus phoxinus)*. Water of moderate hardness was obtained by dilution of the normal water of the town with distilled water. Its characteristics were: pH 7.4; dissolved oxygen, 9.3 ppm; total alkalinity as $CaCO_3$, 115 ppm; and temperature, 18° C (± 1°).

All concentrations are expressed as the active ingredient of the product. Two series of tests were conducted, one in plain water aquaria and another in aquaria containing soil in the bottom and plants *(Callitriche* and *Elodea)*.

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In the first series (without plants) the simazine at a concentration of 0.5 ppm was lethal in less than 3 days for 60 percent of the fish; in the second series (aquaria with soil and plants), however, the concentrations of 2.5 and 5.0 ppm (normally used for this herbicide) were not lethal during a period of 1 month. During this time, the plants disappeared as a result of the action of the herbicide; whereas they remained normal and alive in the control aquarium.

Recently the composition of the Herboxy was slightly changed in the by-products of the simazine (which was always 50 percent in the Herboxy), and new tests have shown that the toxicity is reduced by the change. For example the concentration of 5.0 ppm which was lethal for 90 percent of the fish in less than 40 hours in the first form, was lethal for only 20 percent of the minnows in 3 days in the new form.

2. Atrazine (2-chloro 4-ethylamino 6-isopropylamine S triazine) was tested also at a concentration of 50 percent in commercial herbicides called A 361, two years ago, and Gesaprime, this year.

The A 361 was slightly less toxic than the Herboxy, the concentration of 0.5 ppm being lethal in 3 days for only 20 percent of the minnows. The 48-hour TL_m was 1.25 ppm. In the tests with soil and plants in the aquaria, however, we observed that the A 361 was still dangerous; at the concentrations studied (2.5 and 5.0 ppm) the minnows were killed between 8 and 15 days, whereas there was no mortality during 1 month with the herboxy.

If we compare A 361 and Gesaprime (each of them with 50 percent of atrazine) we observe that the change in the by-products has reduced the toxicity as the TL_m is now 3.75 ppm and the maximum concentration that is non-lethal in 48 hours is 2.5 ppm.

Table 1 shows the results obtained for these products.

3. Weedex, Weedazol, and Weedazol T. L. These herbicides are formed with 2-4D and aminotriazol. They were studied for 1 month in aquaria under the same conditions described above: Temperature, 18° C (± 1°); pH, 7.4; total alkalinity as $CaCO_3$, 115 ppm; dissolved oxygen, between 7 and 9 ppm. The concentrations tested were those normally used: 40 and 80 ppm for the Weedex, 15 and 30 ppm for the Weedazol, and 20 and 40 ppm for the Weedazol T. L.

Because the toxicity of these products is low, the first tests were followed by bioassays conducted in the field. The same numbers of fish were placed in 8 small ponds, each of the same area: 1,000 fry of roach *(Gardonus rutilus* L.) and 500 fry of tench *(Tinca tinca* L.) in the first test, and 100 roaches (weighing 100 to 150 grams) in the second test. After 1 month, each pond was emptied and the fish were counted.

The results of these assays indicate that these herbicides are non-toxic for *Gardonus* when their weight is over 100 grams since in the control pond less than 10 percent of the fishes disappeared during 1 month. They are toxic, however, to young fish; Weedex caused a mortality of 50 percent, and the two Weedazols a mortality of 75 percent of the fry of the roach and the tench.

TOXICITY OF TWO BACTERICIDES

The two bactericides tested contained quaternary ammonium compounds.

1. Sovicide Tetraminol, a cyclic quaternary ammonium in powder with 96 to 100 percent active product.

These tests were limited to 6 hours and the fish were observed for 48 hours after the test. The experimental water was the same as before. The highest concentration non-lethal in 6 hours was 4 ppm, and the lowest concentration at which all fish died was 8 ppm.

Table 1. TOXICITY TO FISH OF PRODUCTS TESTED

Product	Highest dilution non-lethal in 48 hr	Lowest concentration lethal in 6 hr	Lowest concentration lethal in 48 hr	TL_m 24-hr	TL_m 48-hr
Atrazine in A 361	0.5 ppm	10 ppm	5 ppm	5 ppm	1.25 ppm
Atrazine in Gesaprime	2.5 ppm	30 ppm	10 ppm	3.75 ppm	
Simazine in Herboxy 1960		7.5 ppm	1.25 ppm		0.5 ppm
Simazine in Herboxy 1962	5 ppm	Insoluble	Insoluble	Insoluble	Insoluble
Algibiol	7.5 ppm	50 ppm	25 ppm	20 ppm	

2. Algibiol, a mixture of quaternary ammonium compounds.

These tests were limited to 48 hours and we determined "the dose minima mortelle")(lowest concentration at which all fish died) in 6 hours (50 ppm) and 48 hours (25 ppm). The 24-hour TL_m was 20 ppm and the highest concentration at which no fish died was between 5 and 10 ppm.

The experimental water and the conditions for these tests were as described above.

TOXICITY OF COPPER SULFATE WHEN USED AS AN ALGICIDE

We should like to refer to an experiment conducted in the field by L. Mazoit, R. Dreffier, and collaborators. They desired to stop the development of algae in rivers whose water is used for potable water in Paris, because the algae rapidly clog filters. Two reaches were selected on a small stream near Sens (Yonne). The upper reach was used for the control, and the lower one received a solution of copper sulfate sufficient to bring the $CuSO_4$ concentration in the stream to 0.20 ppm.

In 6 days, 75 percent of the carp and tenches were killed, as well as most of the minnows present in the stream (too many to be counted); some of them, however, tolerated 0.30 ppm. Among the fauna, all the larvae of Ephemeropterae were killed while those of Trichopterae remained well, seemed excited, and rushed toward the dead fish.

TOXICITY OF DETERGENTS

The experiments of J. Wurtz-Arlet on the anionic and non-ionic detergents showed their toxicity to the eggs and fry of brown trout and rainbow trout. In her first work she studied the influence of two alkylsulfates, one primary and the other secondary, on the eggs and fry of the brown trout of different ages (24 hours, 3 weeks, and 6 weeks). The conditions of the tests were constant: Temperature, 7° to 8° C; dissolved oxygen, 10 ppm; pH 7.3; total alkalinity as $CaCO_3$, 205 ppm. She reported that:

1. The sodium primary alkylsulfate is less toxic than the secondary alkysulfate for the fry.
2. The eggs with embryos tolerate, without apparent damage, concentrations up to 10 ppm during 24 hours.
3. Fry of 6 weeks are more sensitive than the younger ones.
4. Fry of 6 weeks cannot stand more than one-half hour in a solution with a 10-ppm concentration of active product, and none of the experimental fish can resist 5 ppm for 15 hours.

In a second paper, J. Wurtz-Arlet reports bioassays conducted with a sodium alkylarylsulfonate (anionic detergent) and a non-ionic compound, octiphenol, on the fry of rainbow trout of 10 days and 65 days, and on young trout 4, 8, and 15 centimeters in length. The water used was much softer than in the first experiment; total alkalinity as $CaCO_3$ was 39 ppm, pH 6.9, temperature between 9° and 14° C, and dissolved oxygen about 75 percent of saturation. She determined the highest concentration non-lethal in 1 hour and 6 hours; the lowest concentrations lethal in 1 hour and 6 hours; and the 24-hour TL_m. She reports that:

1. The toxic action is more rapid at concentrations between 15 and 50 ppm for the anionic compound than for the non-ionic compound.
2. At lower concentrations (3 to 5 ppm) the toxicity is almost the same for the two detergents.
3. The 24-hour TL_m is very near the highest concentration non-lethal in 6 hours.
4. The young trout of 15 centimeters seem more sensitive than the younger ones, especially in regard to the anionic compound.

TOXICITY OF SODIUM CHLORIDE

Among the long-term bioassays conducted at our station, were those of P. Laurent who studied the resistance of many aquatic organisms to an NaCl concentration of 10,000 ppm (10 gram per liter). These experiments were conducted to determine the influence of the discharge, in the Rhine, of waste waters from potash mines in Alsace. Among the organisms studied were the Cyprinidae (roach, rudd, and dace), which lived the longest. At a temperature of 23° C, 50 percent of the fish were killed in 10 days, and the other 50 percent in 62 days. When the temperature remained between 7° and 10° C, however, the fish lived for a long time; only 50 percent were killed in 70 days.

Asellus was the second most sensitive form; 50 percent died in 7 days and 100 percent in 48 days. Fifty percent of the *Hydropsyche* died in 6 days and total mortality occurred in 17 days; 50 percent of the *Dressenia* died in 5 days, and there was total mortality in 10 days; and 50 percent of the *Sphaerium* died in less than 2 days with total mortality in 6 days.

P. Laurent found that the same concentration of 10 grams per liter was also toxic to aquatic plants. In 13 days, the plants *(Callitriche, Helosciadium nodiflorum, Oenanthe fluviatilis)* turned yellow and died in salt water, while they lived very well in the control aquarium. Only *Lemna trisulca* lived in the salt solution.

TAINTING OF FISH BY OUTBOARD MOTOR EXHAUST WASTES AS RELATED TO GAS AND OIL CONSUMPTION

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According to figures compiled by the Outboard Boating Club of America, 6,050,000 outboard motors were in use in American waters during 1960. (Shea 1961). There has been a tremendous increase in water sports during the last decade, especially motor boating and water skiing. In areas of intensive motor boat use, it seemed possible that water quality impairment might include tainting of fish.

Laboratory tests (English et al., 1961) showed that bluegills could be tainted by exposure to water contaminated by outboard motor exhaust. Ninety percent of persons comprising a taste panel noted objectionable flavor when the fuel consumption was 8.6 gallons per acre-foot of water. The level at which half of the panel members noted objectionable taste was estimated to be 1.1 gallons of fuel per acre-foot. The gasoline used in the study was regular grade, and the lubricating oil, (1/2 pint/gallon or a ratio of 1 to 17) was a popular brand of packaged outboard motor oil.

These laboratory experiments demonstrated that water from an experimental tank in which outboard motors consumed fuel at the rate of 170 gallons per acre-foot of water killed half the test fish, bluegills and fathead minnows, in 96 hours at a dilution of about 1 in 5. Thus the 96-hr TL_m for water subjected to such use was found to be 19 percent. This is not the safe concentration, but the estimated level at which 50 percent of the fish would die. A factor of 10 was arbitrarily applied for the estimation of a "safe" level, which in this case would be 17 gallons of fuel per acre-foot.

The California Department of Public Health (1961) reported a study to determine the effect of the operation of an outboard motor on water quality. A 10-horsepower motor was operated in 200 liters of water for varying periods of time up to 3 hours. Significant increases in chemical oxygen demand, total solids, lead, phenols, oil, and grease were noted in the laboratory tests, but at reservoirs where boating was popular such materials were not observed in the surface waters. The discrepancies between laboratory and field findings were attributed to the relatively small number of boats on the reservoirs studied and the large dilution in the reservoirs.

The laboratory studies by English et al. (1961) revealed a need for field studies to determine whether fish are tainted or killed by outboard motor exhaust wastes under natural conditions. In 1961 such field studies were carried out cooperatively by the Chemistry and Physics Section and the Aquatic Biology Section of the Basic and Applied Science Branch, Division of Water Supply and Pollution Control of the Robert A. Taft Sanitary Engineering Center.

EXPERIMENTAL AREAS

Field experiments were carried out in three impoundments, Oeder Lake, a nearby sky pond (unnamed), and Eggerding Pond. The main test area, Oeder Lake, which is near Morrow, Ohio, is an artificial impoundment, the main body of which contains 6.89 acres, with an average depth of 11 feet and a volume of 74.8 acre-feet. On August 15, 1961, the lake was stratified at a depth of 15 to 20 feet. Below this level in the deeper part of the lake, the oxygen content was less than 0.5 ppm and at 23 feet the DO was absent. Fish activity was limited, therefore, to those areas less than 15 feet deep during midsummer. Approximately 40 percent of the volume of water was contained in the upper 4-1/2 feet.

The lake is privately owned by a contractor who permitted his employees and friends to use the area for water skiing, boating, fishing, and picnicking. Water skiing was quite heavy through the late spring and summer. The owner cooperated in requesting his boating visitors to record the number of hours they operated boats, the horsepower of motors used, the number of gallons of fuel mixture burned, and whether the gasoline was white or leaded.

The control area was a sky pond on the same grounds as Oeder Lake, less than a half-mile away, in the same general watershed. It is a half-acre sky pond receiving its water entirely from surface drainage from the pasture area above it. Although located below Oeder Lake, it does not receive drainage from it.

The pond remained clear throughout the test period except for plankton growths. It is completely surrounded by fences, which prevent access by livestock. The control pond has an average depth of 6 feet; its volume is 3 acre-feet. An early spring growth of *Potamogeton crispus*, which was quite dense about the shore line when liveboxes were first introduced, fruited and disappeared at the onset of warm weather. There was no boating on the control pond.

A second test pond, Eggerding Pond, was used for operation of outboard motors by project personnel over a known length of time and with known outboard motor fuel consumption. The pond has an area of 0.96 acres, an average depth of 5.4 feet, and volume of 5.2 acre-feet. It is located off Red Bank Road within Cincinnati city limits, on the trucking farm of Eggerding Brothers, less than five miles from the Taft Sanitary Engineering Center. The pond is surrounded by pasture, and the cattle have access to it, but a deep, muddy bottom confines them to the shallow edges.

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* U.S. Department of Health, Education, and Welfare, Public Health Service, Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio.

At the onset of warm weather, floating masses of filamentous algae threatened interference with the operation of outboard motors. The bulk of the algae *(Cladophora)* floated on the surface and was removed by seining prior to placing the liveboxes. In addition, the surface 2 feet of water was treated with 0.3 ppm copper sulfate on June 26 and July 21 to discourage new algal growths.

EXPERIMENTAL CONDITIONS

Adult bluegills *(Lepomis macrochirus)*, 6 to 8 inches long, were placed in each of the ponds in floating liveboxes 2 x 2 x 2 feet, with trap doors, 10 x 10 inches, hinged to the wooden top. The frames of the liveboxes were of 2- x 2-inch white pine; marine plywood was used in the solid wooden top provided to give cover and prevent the stampeding of the fish. The remainder of the box was of 2- or 4-mesh galvanized hardware cloth. Up to 30 adult fish were placed in each livebox.

The fish were fed ordinary white bread every other day. While it is not a balanced diet, bread contains sufficient air to float and sinks at a low rate compared with pelletized, balanced feeds. The fish began to feed upon the bread almost immediately.

The test period extended from June 1 to September 20, 1961, in Oeder Lake and the Control Pond, and from June 29 to September 29, 1961, in Eggerding Pond. At intervals throughout these periods, personnel from the Chemistry and Physics Section collected water samples for threshold odor determinations and for the quantitative determination of oil, lead, carbon chloroform extractables, and chemical oxygen demand. The water quality aspects of this sampling will be the subject of another report. The water analysis data for threshold odor, carbon chloroform extractables, and chlorine demand showed significant increases through the season.

The amount of fuel used on Oeder Lake and Eggerding Pond is shown in Figure 1. The rate of fuel use on the lake was not steady but fuel consumption on a weekly average basis was quite steady through most of the season. The average fuel-use rate is equal to the slope of the lines of cumulative fuel consumption. The collection of data on fuel consumption was begun May 24 in Oeder Lake and July 14, 1961, in Eggerding Pond.

On Eggerding Pond, outboard motors were operated by project personnel and accurate fuel consumption records were maintained. Four outboard motors were used; a 10-horsepower 1960 model, an 18-horsepower 1960 model, a 10-horsepower 1959 model and a 5.4-horsepower model built between 1939-1949. Special propellers were used that allowed the motors to reach optimum operating conditions under fill load yet moved the boat at a very slow surface speed; thus wave action and violent maneuvering of the boat in the small pond were avoided. Six popular brands of regular-grade leaded motor gasoline and outboard-motor lubricating oil were used as fuel. One-half pint of outboard motor oil was added to each gallon of gasoline.

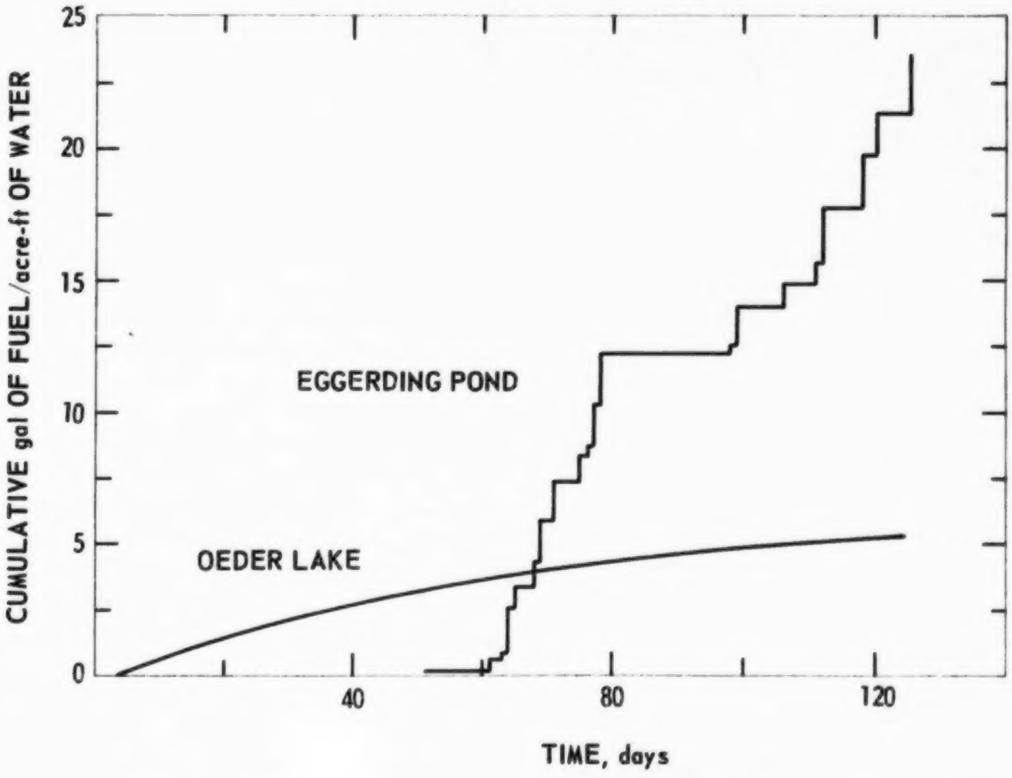

Figure 1. Rate of fuel consumption per acre-foot of water.

STOCKING THE LIVEBOXES

On May 25, 1961, two liveboxes stocked with 30 bluegills each were placed near each other at the surface in the control pond where there was no boating. Three or four fish at a time were removed periodically from these liveboxes for taste tests and no losses occurred until July 20 (after 56 days), when one fish was found dead. In strong contrast to this, severe losses occurred in Oeder Lake where two liveboxes were placed at each of three points on the lake. Between May 25 and June 1 when the boxes were restocked, losses were 50 percent or more. Although the boxes were heavily anchored at the surface to concrete building blocks, losses from fungused fish occurred until the boxes were sunken far enough below the surface (4 to 7 feet) to prevent wave action of passing boats from injuring the fish. This saved enough fish so that the lot stocked in Oeder Lake on June 1, 1961, could be sampled throughout the remainder of the summer.

Because early losses of fish in liveboxes had been heavy, a 500-gallon circular tank was set up on July 11 on the permanent dock on the west side of Oeder Lake. It was stocked with 75 adult bluegills on July 12. Several fish were lost in handling or from injury in attempting to escape from an initially unscreened outlet pipe. The rest of the fish remained in excellent condition for the balance of the test period. Surface water was pumped through the tank at a rate of about 12 to 30 gallons per minute. A carbon filter was inserted in a bypass line for continuous sampling of hydrocarbons and other pollutants in the surface water. This appeared to be an ideal arrangement because the fish could not be damaged by passing boats nor could they be disturbed by the over-curious as the lid on the tank could be locked.

In Eggerding Pond, the adult bluegills were held in liveboxes at the surface as in the control pond, but the fish were not exposed to excessive wave action of motor boats as in Oeder Lake, because outboard motors used in the experiment were provided with special low-pitch propellers that permitted about 4200 rpm and normal fuel consumption without rapid forward propulsion of the boat.

The original two liveboxes were each stocked with thirty 6- to 8-inch adult bluegills on June 29. They lived normally through the first week of August; then on August 11 all fish suddenly died. The kill affected both livebox fish and wild fish in the pond. The outboard motors had been operated heavily that week, but later observations indicated that the kill was probably due to an outbreak of *Cytophaga columnaris*. This bacterial disease appeared immediately upon the restocking of the original liveboxes plus one new livebox on August 16. On August 18, the pond was treated with copper sulfate at 0.5 ppm for an average depth of 5 feet, and the losses ceased.

METHODS OF PREPARING FISH AND SAMPLING BY PANEL

Two methods of cooking the fish were employed, frying and baking. Frying was favored in the early stages of the study and was used throughout because it is the method most widely employed in ordinary kitchen practice; baking was started in late August, and was continued throughout the test period in order to compare the two methods.

After the fish were scaled and head and entrails removed, the fish were rolled in cracker crumbs and fried in vegetable oil in electric frying pans at 380° F until browned on both sides. Usually three fish from the same livebox or lot were fried at a time. During transportation fresh aluminum foil was used to wrap the whole fish. Each fish when cooked was usually divided into four portions, which were wrapped separately in aluminum foil and delivered warm to twelve panel members. The portions were identified by letters and numbers in such a manner that the panel members did not know the body of water nor the lot from which the fish came. Fish from the control pond were always prepared, identified in the same manner, and delivered at the same time as the experimental pond portions. This method is essentially one described by Winston (1959) and used by a large chemical company for many years. It is also similar to the methods used by Davis (1960) except that we did not indicate the control fish to the panel. When we did designate them on one occasion, all panel members recorded 0 (no objectionable flavor); it was felt, however, that an occasional fish might have a muddy flavor or be tainted by natural means.

During the period when the fish were both fried and baked, the cleaned fish were cut in about equal anterior and tail portions. One-half of each fish was fried and the other half baked, and each of these portions was subsequently divided into two portions and delivered warm in aluminum foil to the panel member. All panel members drank cold milk after tasting each portion to remove any flavor remaining from a previous sample.

The fish were baked in aluminum foil for 20 minutes at an oven temperature of 350° F. No seasoning of any kind was added to either the fried or baked fish. Cracker crumbs were not used on the baked fish.

Baldwin et al. (1961) determined both *aroma*, the sensation experienced upon sniffing as opposed to ordinary breathing, and *flavor*, defined as the characteristics observed when food is placed in the mouth and masticated, in a seasonal study of several species of fish from polluted and unpolluted waters. Aman (1955) has pointed out that when we use "taste" we are actually referring to the more inclusive term "flavor." In the true "taste" only sweet, sour, bitter, and salt are detected; "flavor" embraces as well the effect of a substance on the senses of smell and touch.

Dawson and Harris (1951) thoroughly discuss the criteria for selecting good panel members and methods of training. Most of the 12 panel members in this study appeared to have a real interest in the project and had served as panel members the previous year. The scores of the regular panel members were reviewed after several tasting sessions to determine whether any individuals were frequently or consistently off in their judgments. Since no such persons could be singled out, the same people served in subsequent sessions.

Each panel member was furnished a card upon which to record the number of the sample and the date, and to check one of the following observations on each sample:

O - no objectionable taste
— - slightly objectionable taste
+ - strongly objectionable taste

RESULTS AND DISCUSSION

Summaries of the taste panel's observations during this study are given in Tables 1 and 2. The results vary considerably from week to week so that it is difficult to see the over-all picture at any given time. An exception to this is seen in Table 2; the original fish in Eggerding Pond were suddenly killed on August 11, apparently from columnaris disease. The fish used to restock the liveboxes on August 16, 1961, became tainted within 35 days (Sept. 20). At this stage, 66 percent of the fish portions fried in vegetable oil were tainted; wheras 83 percent of those baked in aluminum foil were tainted.

When the results for the total test period were analyzed, "slightly objectionable" and "strongly objectionable" were combined as "objectionable." The results were analyzed statistically by the chi-square test (Snedecor 1956), in which chi-square = $x^2 = \frac{(O - E)^2}{E}$, where O = observed and E = expected (Table 3). Chi-square at probability 0.05 for one degree of freedom equals 3.841. At this level the chances are only 1 in 20 that the differences in number of positive flavor responses for fish from the control and the test ponds occurred by chance alone. This level was arbitrarily selected as the point at which a difference would be accepted as real. The

Table 1. SUMMARY OF THE TASTE PANELS' OBSERVATIONS ON THE TAINTING OF BLUEGILLS IN OEDER LAKE AS COMPARED TO FISH FROM THE CONTROL POND

		No objectionable taste (0)		Slightly objectionable taste (-)		Strongly objectionable taste (+)		Total portions tasted
Date, 1961	Source[a] and cooking method	No. of portions	Percent	No. of portions	Percent	No. of portions	Percent	
June 27	OL-fried	34	94.4	2	5.6	0	0.0	36
	CP-fried	12	100.0	0	0.0	0	0.0	12
July 18	OL-fried	18	50.0	13	36.1	5	13.9	36
	CP-fried	9	75.0	3	25.0	0	0.0	12
Aug. 8	OL-fried	14	58.3	8	33.3	2	8.3	24
	CP-fried	6	50.0	6	50.0	0	0.0	12
	OL(Tank)-fried	7	58.3	4	33.3	1	8.3	12
Aug. 23	OL-fried	12	50.0	8	33.3	4	16.7	24
	OL-baked	1	8.3	2	16.7	9	75.0	12
	CP-fried	10	83.3	2	16.7	0	0.0	12
	CP-baked	8	66.7	4	33.3	0	0.0	12
	OL(Tank)-fried	5	41.7	5	41.7	2	16.7	12
Sept. 8	OL-fried	7	58.3	4	33.3	1	8.3	12
	OL-baked	3	25.0	5	41.7	4	33.3	12
	CP-fried	11	91.7	1	8.3	0	0.0	12
	CP-baked	8	66.7	2	16.7	2	16.7	12
	OL(Tank)-fried	9	75.0	3	25.0	0	0.0	12
	OL(Tank)-baked	7	58.3	2	16.7	3	25.0	12
Sept. 20	OL-fried	9	75.0	2	16.7	1	8.3	12
	OL-baked	4	33.3	5	41.7	3	25.0	12
	CP-fried	9	75.0	3	25.0	0	0.0	12
	CP-baked	9	75.0	2	16.7	1	8.3	12
	OL(Tank)-fried	9	75.0	3	25.0	0	0.0	12
	OL(Tank)-baked	5	41.7	5	41.7	2	16.7	12
	Totals	226	62.7	94	26.1	40	11.1	360

[a]OL = Oeder Lake; CP = Control Pond. Fish were from liveboxes, unless Tank is indicated.

results of both the fried-fish and the baked-fish testing procedures for the entire season were analyzed as a group for each of the test ponds.

The tainting results for fish from Oeder Lake (motor lake) and the control pond that were fried had a chi-square value of 4.4425 (Table 3, A). The probability that the differences occurred by chance is quite low. The overall results with the baked fish procedure that was started late in August, showed a chi-square value of 12.74 (Table 3, B), which indicates that the probability of the differences occurring by chance alone is very remote. The greater value may be due in part to the fact that the fish had been exposed for several weeks to water polluted by outboard motor exhaust wastes before the baking tests were started.

The total results for fish from the 500-gallon tank set up on the dock at Oeder Lake July 11 showed a chi-square value of 1.74 for the fried fish and 3.08 for the baked fish, Table 3 portions, C and D, respectively. Both of these values are below the 3.84 value that was initially adopted as the criteria for a significant difference, but the exposure time was 6 weeks less than for Oeder Lake livebox fish.

In Eggerding Pond (motor pond) where outboard motors were operated a known length of time and fuel consumption was measured, the results were striking in spite of the difficulties encountered in maintaining fish. Table 3 shows the results for the entire season. Bluegills were stocked in the liveboxes in that pond June 29. In a little more than a month, 75 percent of the fish portions tasted by the panel showed some degree of tainting. After columnaris disease or outboard motor exhaust wastes wiped out the initial stock of bluegills in the liveboxes, wild fish seined from the pond with considerable difficulty because of their scarcity, were sampled August 17 and 23. On August 17, 65 percent, and on August 23, 50 percent of the portions of fried wild fish (bluegills outside the liveboxes) were tainted. On August 23, baked portions of the same fish showed 83 percent tainted. Bluegills seined from the pond on June 26, 1962, prior to the operation of outboard motors, and fried showed no tainting.

Table 2. SUMMARY OF THE TASTE PANELS' OBSERVATIONS ON THE TAINTING OF BLUEGILLS IN EGGERDING POND AS COMPARED TO FISH FROM THE CONTROL POND

		No objectionable taste (0)		Slightly objectionable taste (-)		Strongly objectionable taste (+)		Total portions tasted
Date, 1961	Source[a] and cooking method	No. of portions	Percent	No. of portions	Percent	No. of portions	Percent	
Aug. 3	EP-fried	3	25.0	3	25.0	6	50.0	12
	CP-fried	8	66.7	4	33.3	0	0.0	12
Aug. 17	EP-fried $^+$							
	Wild fish [c]	8	34.8	7	30.4	8	34.8	23 [b]
	CP-fried	12	100.0	0		0		12
Aug. 23	EP-fried $^+$							
	Wild fish	6	50.0	3	25.0	3	25.0	12
	EP-baked $^+$							
	Wild fish	2	16.7	6	50.0	4	33.3	12
	CP-fried	0	83.3	2	16.7	0	0.0	12
	CP-baked	8	66.7	4	33.3	0	0.0	12
Sept. 20	EP-fried	4	33.3	3	25.0	5	41.7	12
	EP-baked	2	16.7	5	41.7	5	41.7	12
	CP-fried	9	75.0	3	25.0	0	0.0	12
	CP-baked	9	75.0	2	16.7	1	8.3	12
Sept. 29	EP-fried	6	50.0	2	16.7	4	33.3	12
	EP-baked	1	8.3	1	8.3	10	83.3	12
	CP-fried	8	100.0	0	0.0	0	0.0	8
	CP-baked	8	66.7	4	33.3	0	0.0	12
	Totals	104	52.3	49	24.6	46	23.1	199

[a] EP = Eggerding Pond; CP = Control Pond. Fish were from liveboxes, unless otherwise indicated.

[b] Observation missing.

[c] Wild fish in pond sampled when columnaris disease killed all fish held in liveboxes and most of the fish in the pond. Liveboxes restocked Aug. 16, 1961, with adult bluegills.

Table 3. RESULTS OF CHI-SQUARE ANALYSIS OF THE TASTE PANELS' OBSERVATIONS ON THE TAINTING OF BLUEGILLS (held in liveboxes) BY OUTBOARD MOTOR EXHAUST WASTES

(Significant chi-square at probability P-0.05 with one degree of freedom - 3.84)

	Total portions untainted		Total portions tainted		Total portions tasted	Chi-square observed
	Observed	Expected	Observed	Expected		
A. Oeder lake (motor lake) compared to control pond (fried)						
Control pond (no motors)	57	50.3	15	21.6	72	
Oeder lake	94	100.7	50	43.2	144	
Totals	151		65		216	4.425 [a]
B. Oeder lake compared to control pond (baked)						
Control pond	23	15.5	13	20.5	36	
Oeder lake	8	15.5	28	20.5	36	
Totals	31		41		72	12.74 [a]
C. Oeder lake tank compared to control pond (fried)						
Control pond	36	33	12	15	48	
Oeder lake	30	33	18	15	48	
Totals	66		30		96	1.74 [b]
D. Oeder lake tank compared to control pond (baked)						
Control pond	17	14	7	10	24	
Oeder lake tank	11	14	13	10	24	
Totals	28		20		48	3.084 [b]
E. Eggerding pond (motor pond) compared to control pond (fried)						
Control pond	47	30.4	9	23.4	56	
Eggerding pond	27	38.6	44	29.6	71	
Totals	74		53		127	20.80 [a]
F. Eggerding pond compared to control pond (baked)						
Control pond	25	15	11	21.5	36	
Eggerding pond	5	15	31	21.5	36	
Totals	30		42		72	22.67 [a]

[a] Significant values.
[b] Not significant. Tank fish exposed for period of 6 weeks less than livebox fish in Oeder Lake.

The chi-square test applied to the total fried portions of fish taken from inside or outside the liveboxes in Eggerding Pond showed a high chi-square value of 20.8 where significance at the 95 percent confidence level was 3.84 for one degree of freedom (Table 3, E). When the fish were baked, even more striking results were obtained (Table 3, F), with the highly significant chi-square value of 22.67.

The level of fuel consumption per acre-foot of water at which fish flesh tainting becomes evident is probably influenced by the daily average rate at which fuel is used in motors operated on the pond. Figure 1 shows that the fuel use rate in Oeder Lake was fairly steady throughout the season; the fuel consumption rate for the two operating periods in Eggerding Pond was also uniform when considered on a weekly basis. Thus tainting results at two fuel use rates are available. At the fuel use rate in Oeder Lake the first indications of tainting occurred between the consumption of 2.2 and 3.5 gallons of fuel per acre-foot of water. At the fuel use rate in Eggerding Pond fish flesh tainting was severe at the first observation made; fuel consumption up to this time was 5.9 gallons per acre-foot.

An estimate of the threshold level of fuel consumption for tainting was made as follows. All tests of the fried control fish averaged 18 percent positive responses. A graph was made by plotting the percent positive responses in fish tainting tests against cumulative fuel use. The positive baseline level of 18 percent for the control was found on this graph to correspond to a fuel consumption of 2.6 gallons per acre-foot of water in Oeder Lake. This appears to be the best estimate that can be made of the level at which motor use should be restricted to avoid fish tainting; it is considered applicable to a rate of fuel use that consumes this quantity over a 2-month period.

The hours of operation and fuel consumption rate of two sizes of outboard motors required for significant tainting of fish are illustrated in Table 4.

Table 4. HOURS OF OPERATION OF OUTBOARD MOTORS (per acre foot of water) REQUIRED FOR SIGNIFICANT TAINTING OF FISH

	Estimated fuel consumption rate, gal per hour		Hours of operation, in acre feet of water, for 2.6 gal of fuel use	
5 HP	1/2		5.2 hr	
50 HP	5		0.52 hr	
	Extreme		Critical	
Water surface area per boat, sq ft	0.92 40,000 (acres)		5.7 250,000 (acres)	
Depth of water, ft	5		10	
Motor operation per day, hr	2		2	
Fuel consumption, gal per hr	1/2	5	1/2	5
Period, days	90	90	90	90
Gal of fuel per acre ft	19.6	196	1.6	16

Because of the possibility that the outboard motor exhaust wastes might be injurious to the red blood cells, examinations for haemoglobin were made of the blood of bluegills from both the control and experimental ponds. These examinations failed to show a significant trend.

ACKNOWLEDGMENTS

Mr. Lester Oeder, Morrow, Ohio, permitted the use of Oeder Lake and a farm pond on his property for the conduct of this experiment. The Eggerding Brothers, Red Bank Road, Cincinnati, consented to the use of their pond for the experimental operation of outboard motors.

The following persons of the Robert A. Taft Sanitary Engineering Center staff served on the taste panel of 12 persons or were "standby" for the regulars: Dr. Louis Williams, Dr. Clarence Tarzwell, Dr. C. Mervin Palmer, Mrs. Carol Scott, Julia Air, Albert Katko, Mrs. Eva Morgan, Thomas Maloney, Mary-Ellen Morris, Quentin Pickering, Ernest Robinson, Mrs. Edith Smith, Robert Davis, Edwin Barth, Richard Carnes, Dr. Donald Mount, Gerald McDermott, and John English.

Julia Air and Mrs. Eva Morgan served as cooks during the project and Quentin Pickering and Richard Carnes seined fish and prepared them for cooking.

Dr. Donald Mount made the haemoglobin tests on samples of fish from the control and experimental ponds.

Dr. Eugene K. Harris, Chief, Statistical Services, reviewed the report and offered suggestions.

Special acknowledgment is made of the work of Robert Breinich, a promising student at Villa Madonna College, Covington, Kentucky, and a hard-working and faithful assistant on this project up to the time of his untimely death.

Adult bluegills for these tests were obtained through the cooperation of the Ohio Department of Natural Resources, Division of Wildlife; the Kentucky Department of Fish and Wildlife Resources; and Dr. Charles Hauser of Glendale, Ohio.

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EFFECTS OF OIL POLLUTION ON MIGRATORY BIRDS

Ray C. Erickson *

Among the factors adversely affecting the welfare of migratory birds is oil pollution, a spectacular and frequently observed cause of bird mortality. Much has been written about these losses, but most reported instances of oil-caused mortality have been based on localized or short-term observations. No comprehensive annual surveys of migratory bird casualties to oil pollution have been made for the entire coast of the United States.

Among the more local or short-term studies, Tuck (1960) included an excellent report of the effects of oil pollution on oceanic, migratory birds along the coast of Newfoundland. Miller and Whitlock (1948) described waterfowl mortality from oil contamination on the Detroit River, while about 10 years earlier, Moffitt and Orr (1938) reported similar losses with waterfowl and other migratory birds in the San Francisco Bay region. More general but nevertheless valuable statements on the overall problem of oil contamination and migratory birds have been presented by Lincoln (1936), Belt (A report to The International Committee for Bird Preservation, "Oil Pollution", dated October 27, 1953, London, England), Giles and Livingston (1960), Hawkes (1961), and U.S. Coast Guard (1961). Other published articles of importance in understanding the problem, but not primarily concerned with the effects of oil pollution on migratory birds and other wildlife, are by California State Water Pollution Control Board (1956, 1959), Gutsell (1921), Lane et al. (1925), Galtsoff (1936), Galtsoff et al. (1935), Gunter (1959), and Dennis (1959: 1960a; 1961). Although the oil pollution problem is largely man-made, natural oil seepages have been reported off " . . . the southern coast of California, in the western Gulf of Mexico, off the eastern coast of Texas and Mexico, along the coast in parts of western and northern Cuba" (Dennis, 1959).

The fossilized remains of prehistoric animals in the natural asphalt deposits at Rancho La Brea, California, show that oil-caused wildlife mortality is not a new problem. Widespread losses did not occur, however, until well into the present century when petroleum fuels became more extensively exploited for oil-burning ships, automobiles, locomotives, airplanes, stoves, and furnaces; black-top roads, and many other uses. Oil may become a mortality factor when spilled into wildlife environments at the site where it is extracted from the ground or anywhere along the line to its final destination and use.

Nearly every body of water on earth, fresh or salt, is occupied by migratory birds at one time or another. The potential for losses of migratory fowl thus exists almost everywhere that surface waters are contaminated by oil. In North America, greatest losses of migratory birds to oil pollution have been observed along the coast and in offshore waters of the Atlantic Ocean and, to a lesser extent, the Gulf of Mexico and the Pacific Ocean. Losses have been particularly heavy in or adjacent to the principal harbors and oil refineries of the Northeast and along the more heavily traveled shipping lanes. The problem has also been encountered in various inland waterways including the Great Lakes. This region is being watched with some concern because of the opening of the St. Lawrence Seaway in 1959 to international deep-draft shipping (Dennis, 1960b).

SCOPE OF PROBLEM

Oil contamination in habitats utilized by migratory birds may be detrimental at any time of the year. Apart from the aesthetic damage, losses may occur during the colder months when greatest numbers of birds are present, or during the growing season when oil may blanket the shores or bottom and reduce or eliminate important sources of food. It is the oil spillage during the seasons and in the areas of heavy migratory bird use that brings about the spectacular losses reported in the press. Because spillage often occurs in or near human population centers along the coast, especially in bays and harbors, losses to coastal dwelling wildlife are much more likely to be observed than mortality of offshore pelagic forms that come in contact with oil out at sea and die unnoticed. Birds that are year-round residents along the coast would appear to be highly vulnerable, for they must survive a much greater period of direct exposure to oil each year than those that appear briefly as migrants.

It is extremely difficult to assess the importance of oil pollution-caused mortality in most wildlife populations. This is particularly true with the very mobile forms. Movements of population units through a contaminated location and various other factors make it difficult to measure what proportion of the total population has come in contact with oil, and, of the segment that has, what proportion is lost.

It is almost certain that relatively few of the birds that contact oil and die far out to sea are observed. The proportion that ultimately drifts to the coast and is seen depends upon air and water currents, the period during which the carcasses remain afloat and on the beach, frequency of surveillance of drift materials, and other factors. Estimates of mortality based on observed remains of oil-soaked birds, ordinarily have value, therefore, only as minimal indications of loss, because the elements that singly or in combination operate to obliterate the evidence may be extremely varied in their ultimate effects. Because many other mortality factors face migratory birds, especially those species that must survive semiannual flights and an annual hunting season, the importance of pollution-caused mortality in the overall scheme of management and preservation of some species is difficult if not impossible to assess accurately. Wide dispersal of a population may spread the risk and be safer than concentration of the entire species in a limited location. Also, mobility and versatility in food acceptance may allow a species to use alternate habitats when preferred environments are contaminated.

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EFFECTS ON BIRDS

Large numbers and varieties of birds are involved in the oil pollution problem. Most, if not all, of the migrants that alight on the open sea, that feed or rest in coastal bays or estuaries and on inland waters, or that move up and down the beach are subject to oil contamination. Birds may also become oiled in borrow pits adjacent to freshly surfaced highways, or on polluted ponds associated with oil wells, pipe lines, and refineries. The possibility has been suggested that birds such as ducks or gulls are attracted to oil slicks because the quieting effect of the slicks on choppy waters may falsely suggest the presence of submerged vegetation or shoals of fish (Tuck, 1960). Since it has not yet been demonstrated that birds actually are attracted to oil, it may be that the contamination of migratory birds occurs when they are drawn to the areas for feeding, resting, or other purposes.

Among the birds most commonly affected are common and red-throated loons, greater scaup, eiders, scoters, oldsquaw ducks, American goldeneyes, various gulls, common and thick-billed murres, and razorbill auks, though other species of loons, grebes, shearwaters, fulmars, petrels, ducks and geese, terns, other shorebirds, auks, dovekies, murrelets, guillemots, and puffins are included on the casualty list. In general, records of oil pollution casualties show a relation between the amount of time that migratory birds spend in the water and frequency of contamination. As examples, murres spend most of their time in the water when not at the nest and are subject to extensive losses, while gulls, though extremely abundant in harbors and other coastal areas where oil pollution is common, seem better able to withstand the adverse effects. This may stem from their inclination to spend much of their time away from water or in the shallows where their feet, rather than their plumage, are more likely to come in contact with oil (Moffitt and Orr, 1938), or their ability to regurgitate ingested materials. On the other hand, some observers believe that gulls are inherently better able to withstand actual fouling by oil than are most other types of migratory birds.

The direct effects of oil on birds depend upon a variety of factors, including the type of oil, extent of contamination of plumage, temperature of air and water, and probably the quantity of oil ingested. In general the heavier oils are a greater problem than the more volatile and are readily dispersed types. Even small quantities of oil may destroy the waterproof qualities of plumage and permit chilling by water or air, especially during the coldest months. The oil may not only saturate the outer contour feathers, but also penetrate the downy layer of ducks and other birds that have this natural insulation. Soaked with oil and water, the bird loses its buoyancy and ability to fly, and can no longer feed effectively. Continued exposure to the cold and reduced ability to obtain food or depart for more habitable surroundings, render the bird incapable of maintaining body temperatures and surviving.

It should not be inferred that all birds coming in contact with oil are doomed. On the contrary, the number that die may be considerably smaller than the number that survive. In areas of chronic oil pollution in the Northeast, hunters are reported to have encountered "oil-burn" on as many as half of the scoters they bagged. One game management agent of the Bureau of Sport Fisheries and Wildlife reported finding extensive, heavy oil contamination on the plumage of 5 percent of all scaup in the hunter's bags he examined. Many hunters are reluctant to use oil-stained birds for food and may throw them away or not even bother to take them into possession because of the actual or imagined tainting of flesh. Because oil often is ingested in the preening process, palatability of contaminated ducks may be reduced, especially if the birds are not dressed soon after being shot. Furthermore, if their feeding activities have been hampered, they may be in poorer flesh and less desirable for the table than birds that have not encountered oil.

Little is known about the toxic effects of ingested oil on migratory birds, except that the alimentary tracts of some victims have become literally lined with residues. Under these circumstances, they usually have been in poor flesh. The rate at which the digestive system is cleared by voiding this material is not known, but most birds that have swallowed oil have little else in their stomachs.

Migratory birds are indirectly affected by deposits of oil on the bottom in shallow water or along the shore that reduce the available food supply of both plant and animal matter. Various elements in the food chains are eliminated by chemical and physical properties of the oil, or items of the diet may be made unavailable by being overlaid or imbedded in tarry materials. The use of important feeding grounds has declined greatly after pollution by oil, either because of the elimination of essential food and cover, or because fouling of the habitat in other ways has reduced the attractiveness of the areas. Shorebirds that rely upon the intertidal zones for their feeding grounds may find them completely blanketed with oil.

It seems likely that oil poses the greatest hazard to wildlife at the time it escapes or is spilled into aquatic and marine habitats. As it continues to be exposed to the action of water, air, bacteria, and other agents, it loses certain volatile components. The remaining, increasingly viscous residue may eventually take the form and consistency of a firm, asphalt-like matrix containing shells, sand, and other debris. Dennis (1959) has classified oil forms into five types, and has indicated that some oils progress more rapidly than others to the solid stage. Oils, when firm, no longer adhere to the plumage of birds. In the more stable and durable stages, oil residues may last for months or even years, but with reduced likelihood of adversely affecting migratory birds. Accumulations of petroleum sludge may, however, prevent germination and growth of plants and the production of invertebrates important as food, either by blanketing the bottom or by toxic effects.

DISCUSSION

Although the oil pollution problem is not new, little intensive study of its ultimate effects on migratory bird species and populations has been made. Oil pollution affects different species in various ways depending upon the extent of the polluted area, and the habits, numbers, and distribution of birds using the contaminated habitats. It is likely to pose a special hazard for species that occupy, during any part of the year, a very limited range susceptible to oil pollution. Two noteworthy examples of this type of vulnerability are the greater snow goose, which winters on the Outer Banks of Virginia and North Carolina, and the whooping crane, which winters almost exclusively in the Arkansas National Wildlife Refuge and vicinity on the Gulf Coast of Texas.

With abundant and widely distributed forms, oil pollution may serve as an agent of intermittent but continuing attrition, especially on those migratory birds using coastal and offshore waters. Most pollution-caused losses occur during the coldest months when the birds are abundant and are concentrated on stopover points or on the wintering grounds. Losses under these circumstances are especially important because they involve the birds that have already survived the southward flight and hunting season and that have become the potential breeding stock for the next nesting season.

Because of the importance of oil pollution from the standpoint of wildlife losses, and from aesthetic and economic considerations, much public interest has arisen concerning what may be done about it. Obviously, eliminating sources of pollution would solve the problem. Ratifying and strictly enforcing the provisions of the 1954 International Convention for the Prevention of the Pollution of the Sea by Oil as amended in 1962, and of existing regulations on oil pollution in domestic waters under the jurisdiction of the nations of North America would be another step in this direction, at least insofar as our native migratory birds are concerned. Even with strict enforcement and observance of such regulations, it is unlikely that complete cessation of oil pollution will result, because shipping accidents, oil line or installation defects, and other human error and equipment failure factors will undoubtedly continue. Enactment and enforcement of stricter laws by States and local governments are especially important, since a 1960 survey showed a lack of effective local laws to be a particularly weak link in the problem of oil pollution control.

Through the years, various methods have been tried for eliminating oil slicks, including the use of carbonized sand, solvents, emulsifiers, detergents, and burning. Some of them have had value for neutralizing or preventing the spread of local or semi-contained foci of contamination. Ordinarily, however, the oil disperses rapidly to form an extensive, thin film. After this has occurred, treatment requires extensive and costly coverage, so that the only complete solution consists in preventing the initial release of the oil by good seamanship, preventive maintenance, and strictly observing regulations for releasing pollutants, including effectively treating oily wastes. To out knowledge, no economical yet efficient technique is available for eliminating sludge deposits that may develop where waste oil has settled, though modest success has been reported on the use of emulsifiers in Great Britain.

Action to reduce the adverse effects of oil pollution at the point of damage to wildlife admittedly is not a satisfactory solution to the problem though it may help to save some birds, permit the protection or rehabilitation of habitat, and provide the basis for participation of private individuals and organizations interested in the humanitarian aspects of this work. Unfortunately, by the time that contaminated birds can be captured, many, if not most of them, are beyond assistance because of external injury, toxic effects, exposure, or starvation. It is not economically feasible to patrol, with sufficient frequency and thoroughness to pick up contaminated birds in time to save them, all the vast expanses where migratory birds may encounter oil. Also, the decontamination and rehabilitation processes now in use are rather laborious and time-consuming, so that costs of treatment remain high.

Many methods of removing oil or tarry deposits from waterfowl plumage have been tried. Various solvents have been in use for more than a score of years. Although the oil may be removed, considerable irritation of the skin usually results and loss of natural oils from the plumage often requires retaining the bird a week or more in captivity before it can be safely released. Soap and water is ineffectual under most circumstances. Use of a "dry shampoo" of Fuller's Earth or powdered chalk has been described by Mrs. Catherine Tottenham of North Devon, England (letter of January 25, 1958 to Dr. Frederick C. Lincoln, U. S. Fish and Wildlife Service). With especially difficult cases, she preceded the Fuller's Earth treatment with a liberal application of butter on the plumage, a warm, soapy bath, and overnight drying. In his letter of June 30, 1952, to Mr. Francis B. Schuler of the Bureau of Sport Fisheries and Wildlife, Dr. Oliver H. Hewitt, of Cornell University, reported a similar method suggested by Dr. D. F. Steadman, involving three formulas of nontoxic ingredients including corn oil, neat's-foot oil, oleic acid, paraffin, a solvent, two commercial detergents with the trade names "Span 40" and "Tween 40", and water. These preparations were used successfully to decontaminate ducks that became severely oiled in 1948 when a tanker ran aground in the St. Lawrence River. They were applied, then wiped off after a minute of two, followed by successive applications as necessary. The birds were kept 10 days before release to the wild fully recovered.

NEEDED RESEARCH

There are several promising lines of wildlife research that should be considered from the standpoint of obtaining a more accurate appraisal of the significance of the effects of oil pollution on migratory bird populations and their habitats, and in the development of more efficient methods of decontaminating the birds and their environments.

(1) An initial step would be the development of a systematic surveillance of sites of chronic oil pollution, as well as of accidental and unusual releases of oil in areas of intensive use by migratory birds, coupled with stricter enforcement of existing laws. Information from this survey would be useful in pinpointing sources of pollution important to these birds and might help to indicate the significance of the impact of oil contamination on this resource.

(2) Study of the behavior of migratory birds in the vicinity of oil spills, accidental or experimental, and development of methods for preventing birds from alighting in the contaminated areas. The use of frightening devices such as floating rotating beacons, exploders, flashers, dyes, and other methods found effective in prevention of waterfowl depredations should be tested.

(3) Further development and testing of methods of decontaminating and reconditioning wildlife suffering from contact with oil pollution, with special attention to techniques that might be used by private citizens or organizations having a minimum of materials, equipment, facilities, and training.

(4) Studies of the short- and long-term effects of oil pollution on plants and animals important to migratory birds and other wildlife and fish as food or cover. Determination of the mechanics of damage and the extent to which alteration of the habitat modifies its use and value to wildlife.

(5) Development and testing of techniques of reconditioning wildlife habitats that have been subjected to oil pollution, as by changes in water management practices, mechanical and chemical decontamination methods, and vegetation plantings, followed by evaluations of wildlife response.

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FACTORS THAT AFFECT THE TOLERANCE OF FISH TO HEAVY METAL POISONING

Richard Lloyd *

INTRODUCTION

A critical review of the literature on the toxicity of the heavy metals to fish, compiled by Doudoroff and Katz (1953), showed clearly that there were considerable differences among the concentrations of a single metal found by different authors to be toxic to fish. It appeared that differences in the test conditions, such as the nature of the dilution water, the duration of the test, and the species of fish used, contributed to the wide variation in the results. Factors such as temperature and the concentration of dissolved gases and calcium in the dilution water had been shown to modify the amount of heavy metal required to kill fish. These experiments had, however, shown only the qualitative importance of these factors; there were few quantitative results.

If the results of toxicity tests made in the laboratory are to be applied to the control of stream pollution, the effects of the important environmental variables found in natural waters have to be quantitatively assessed. This paper reviews some of the work done at the Water Pollution Research Laboratory, England, on this problem.

MODE OF ACTION OF HEAVY METALS

Many early workers attributed the toxicity of heavy metals to the precipitation of mucus on the gills of the fish, but it was later suggested that the gill epithelium might be damaged by the formation of insoluble metal-protein complexes. Histological examination of the gills of rainbow trout killed in solutions of zinc, copper, and lead salts at this laboratory showed that the epithelial cells became swollen, separated from the pilaster cells of the lamellae, and were finally sloughed off. A similar observation was made by Schweiger (1957) using carp as the test fish in solutions of heavy metals. In other tests, Parry (in Lloyd, 1960) found no trace of precipitated mucus on the gills of rainbow trout killed by zinc poisoning.

Analysis of the tissues of rainbow trout killed by a solution of zinc, containing Zn^{65} as a tracer, showed that the gill tissues contained the highest percentage of zinc (Lloyd, 1960), although radioactive zinc was found in other parts of the body. Saiki and Mori (1955) and Joyner and Eisler (1961) have shown that zinc can be absorbed into the body of the fish from sublethal concentrations in the water. Schweiger (1957), however, took carp killed by solutions of cobalt and manganese and determined the concentrations of these metals in the body, then injected healthy carp with either three times that amount of cobalt or three hundred times the amount of manganese; the fish survived. This suggests that the toxic action is not internal and that, at least for some heavy metals, it is confined to the epithelial cells of the gill lamellae. Analysis of the gills of rainbow trout killed by a zinc solution showed that the concentration of metal present was only double the normal zinc content, so that the amount required to kill may be quite small.

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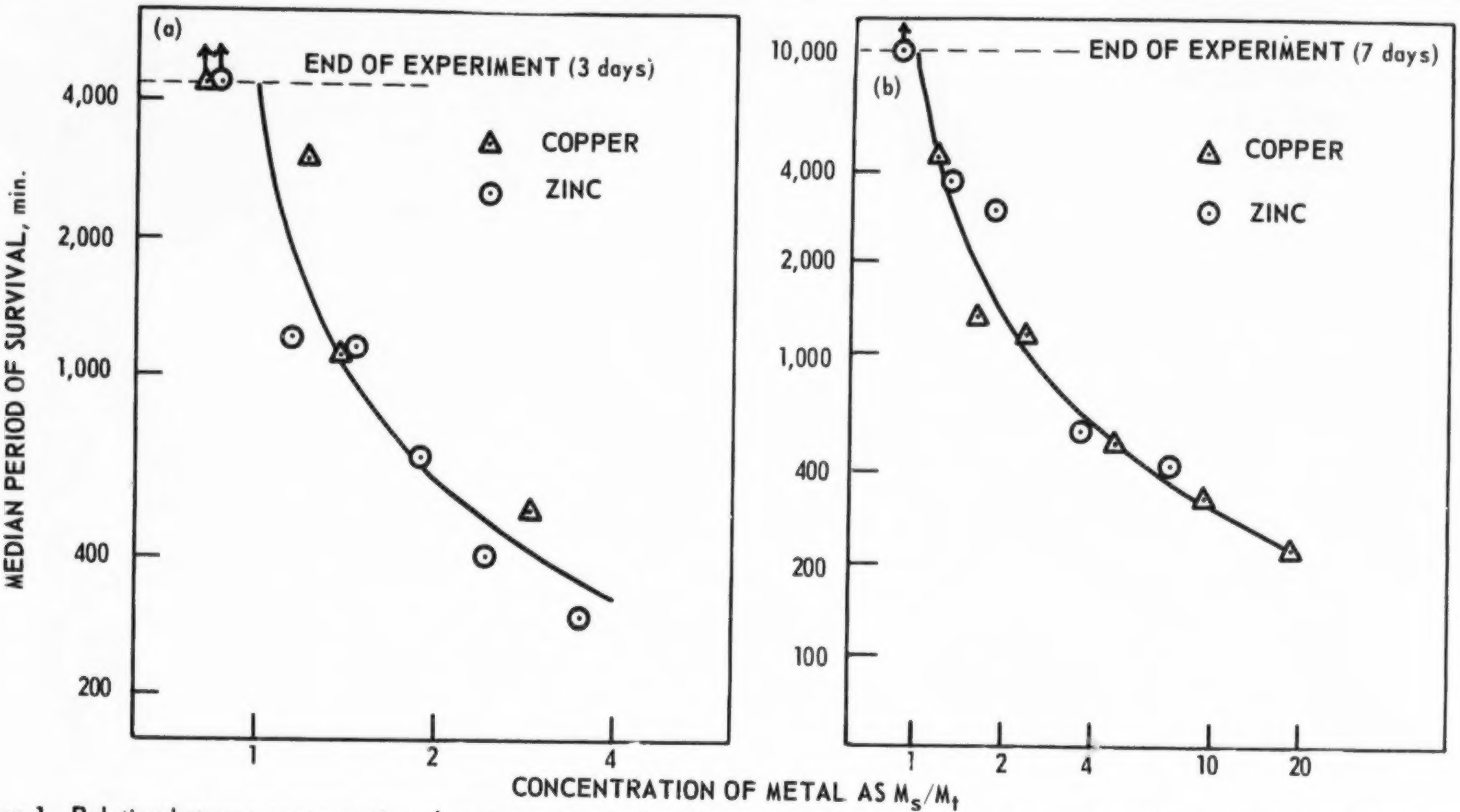

Figure 1. Relation between concentration of copper or zinc and median period of survival of rainbow trout, (a) in hard water, (b) in soft water. For explanation of M_s/M_t see text.

If the toxic action of heavy metals is confined to the gill epithelium, then the following hypothesis can be put forward. When the rate at which the heavy metal ions enter the gill epithelial cells is less than the rate at which they are removed into the blood stream, no build-up of metal ions will occur in the epithelial cells, and the fish will survive. If the rate at which the heavy metal ions enter the gill epithelium is greater than the rate at which they are removed into the blood stream, then a build-up will occur, and the fish will die. This suggests that there will be a lethal threshold concentration of heavy metals for fish. When this concentration is present at the gill surface, the rate at which the metal enters the gill epithelial cell is balanced by the rate at which these ions are removed into the blood. Inspection of the log concentration-log survival time curves for several heavy metals indicates that a lethal threshold concentration probably exists, since after a period of some days these curves become parallel with the survival-time axis.

If the concentrations of the heavy metals are expressed as the multiple of their lethal threshold concentrations, i.e., M_s/M_t, where M_s is the concentration of metal in solution and M_t the lethal threshold concentration, the log concentration-log survival time curves for copper and zinc salts to rainbow trout are identical, both in hard and soft water (Figure 1), indicating that their toxic action may be similar. If Schweiger's (1957) data for the toxicity of cadmium, nickel, cobalt, mercury, and manganese to rainbow trout are re-plotted in this way (Figure 2), they show that, although the points for the first three metals lie on the same curve, those for mercury and manganese are different, which may indicate that these two metals have a somewhat different action.

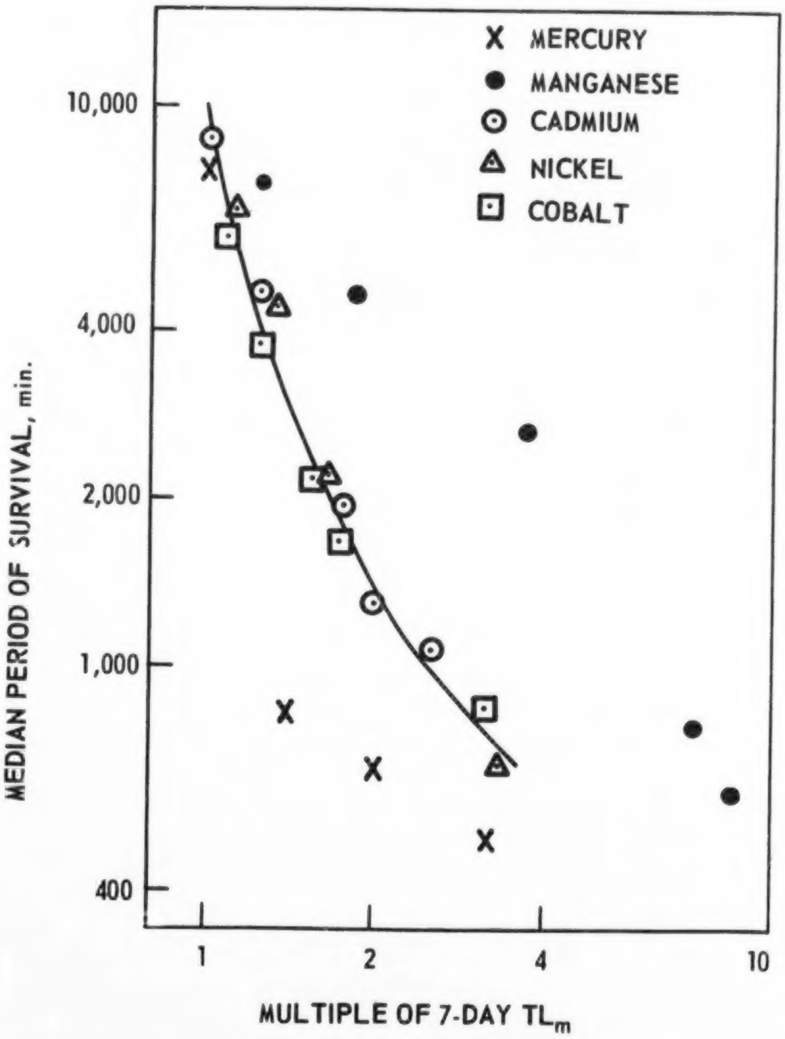

Figure 2. Relation between concentration of several metals and survival time of rainbow trout (data from Schweiger, 1957).

EFFECTS OF ENVIRONMENT ON TOXICITY

A change in the susceptibility of a fish to heavy-metal poisoning can be measured either by a difference in survival time or by a difference in the concentration of metal required to kill the fish in a given time. Because of the curvilinear shape of the log concentration-log survival time curve, the most useful method is to compare concentrations of metal required to kill the fish only after a considerable exposure time, and the optimum time is that at which the TL_m obtained is a lethal threshold concentration.

CALCIUM CONCENTRATION

Although many heavy metals have been shown to be more toxic in soft water than in hard water (Jones, 1938; Tarzwell and Henderson, 1955; Cairns and Scheier, 1957; Lloyd and Herbert, 1962), the extent of the difference in toxicity appears to vary for different metals. Some of the differences observed may have been caused by the decreasing solubility of the heavy metals as the water becomes more alkaline, but the presence of calcium ions in the water also reduces the toxicity of heavy metal ions. With rainbow trout there is some evidence (Lloyd and Herbert, 1962) that, when the log lethal threshold concentrations of either zinc, copper, or lead salts in solution are plotted against the log total hardness of the water, the points obtained are adequately fitted by a straight line (Figure 3) from which the lethal threshold concentration for any intermediate total hardness value can be quite accurately interpolated. It is interesting to note that the slopes of the lines obtained are different for each metal.

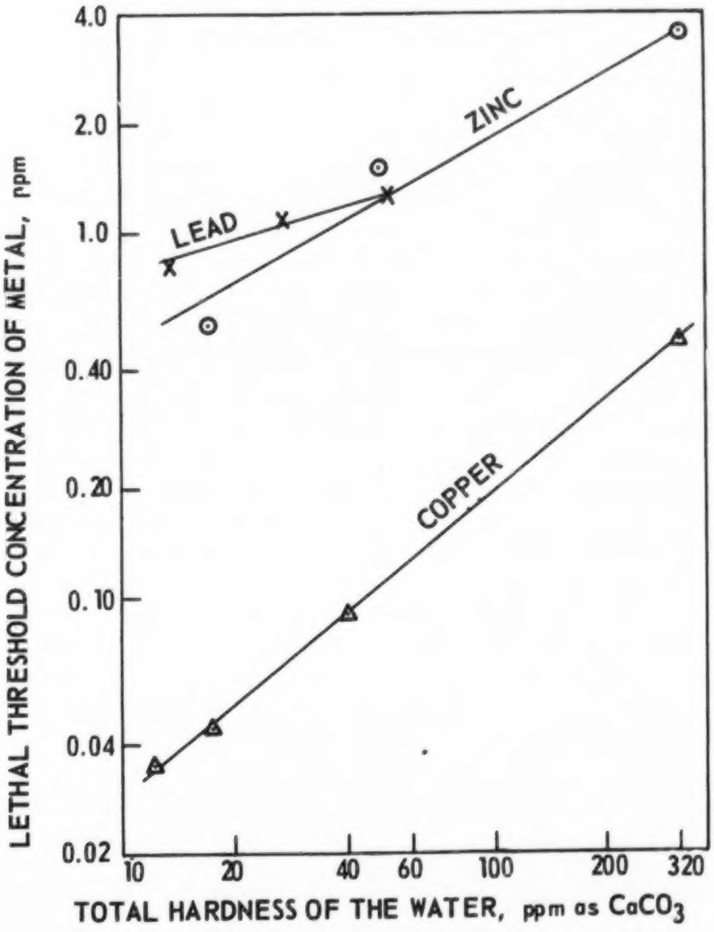

Figure 3. Relation between the total hardness of the water and the lethal threshold concentrations of dissolved lead, zinc, and copper salts for rainbow trout.

It is not known why an increase in calcium ion concentration reduces the toxicity of heavy metals, since Jones' (1938) conclusion that the presence of calcium prevented the precipitation of mucus does not hold for rainbow trout, where no precipitated mucus was observed. Some experiments made at this laboratory, however, may shed some light on the problem. Preliminary experiments on the effect of water hardness on the toxicity of zinc were made with rainbow trout reared in hard water, and batches were acclimatized to softer waters for up to 3 days. Under these conditions the toxicity of zinc to rainbow trout was similar in both hard and soft water. Further experiments showed that rainbow trout reared in soft water were more sensitive to zinc poisoning in soft water than those reared in hard water and tested in soft water. Finally, it was found that rainbow trout that had been kept in hard water had to be acclimatized to soft water for a least 5 days before they became as sensitive as soft-water reared rainbow trout. This suggests that the protective action of calcium is internal and that some calcium has to be lost by the hard-water reared fish before they attain soft-water sensitivity. Some support for this suggestion is given by Houston (1959) who has shown that the cellular content of calcium in the steelhead trout may increase with the calcium content of the water.

EFFECT OF TEMPERATURE

A decrease in temperature usually increases the survival time of fish in toxic solutions; experiments with rainbow trout in solutions of zinc (Lloyd, 1960) showed that the survival times increased 2.35 times when the temperature was reduced from 22° C to 12° C. If temperature affected only survival time, then the lethal threshold concentration would be unaffected by temperature, since at this concentration survival time curve is parallel to the survival-time axis. The data in Figure 4 show in fact that this is so.

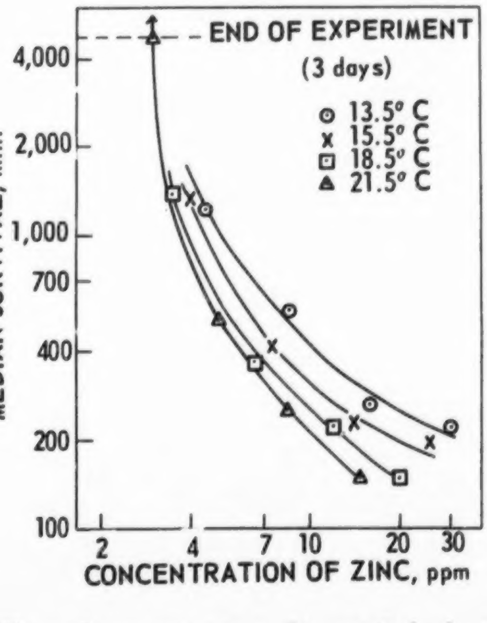

Figure 4. Effect of temperature on the survival of rainbow trout in solutions of zinc.

A similar conclusion was reached by Cairns and Scheier (1957) for the toxicity of zinc to bluegills, since there was little difference in the 96-h TL_m values obtained at 18° C and 30° C in either a hard or a soft dilution water.

DISSOLVED-OXYGEN CONCENTRATION

The increase in toxicity of a poison, which occurs when the dissolved-oxygen concentration of the water is reduced, can be expressed by dividing the lethal threshold concentration obtained with oxygen at the air saturation value (X_{sat}) by the lethal threshold concentration obtained for a lower oxygen concentration (X). The resulting fraction, X_{sat}/X, can then be plotted against the oxygen concentration of the water. It was found (Lloyd, 1961 a) that the curves so obtained for zinc, lead, and copper salts, with use of rainbow trout, were similar in form (Figure 5). Futhermore, curves obtained for phenol and, with some modification, ammonia were also similar to those obtained for the heavy metals (the curve drawn in Figure 5 takes into account the data obtained for these two poisons).

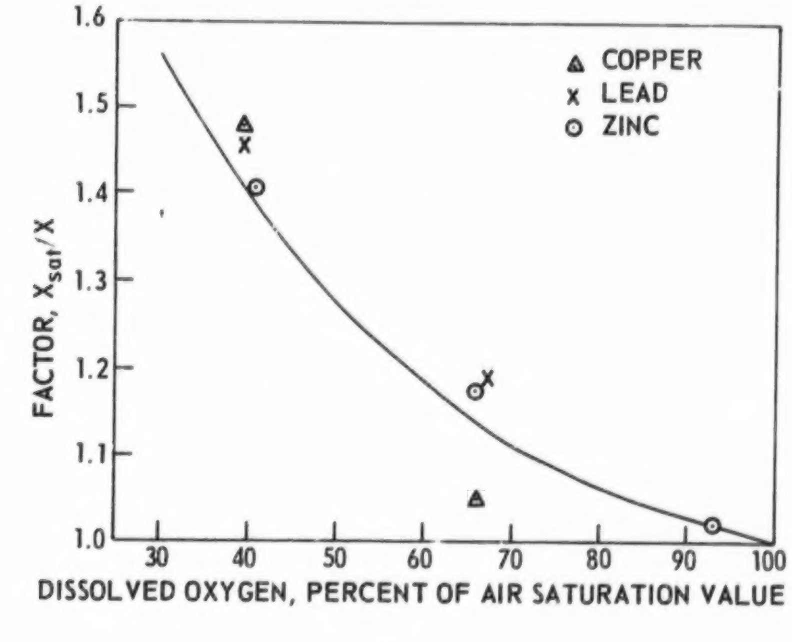

Figure 5. Relation between the factor X_{sat}/X for copper, lead, zinc and the dissolved-oxygen content of the water. For explanation of X_{sat}/X see text.

Since the toxic actions of the heavy metals, phenol, and ammonia were different, it appeared that the increase in toxicity was owing to a physiological response by the fish to water of low oxygen content, and that the most obvious response was pumping water more rapidly through its gills.

The following hypothesis has been put forward to explain why this increase in respiratory flow also increases the toxicity of poisons. It is known that the rate at which ions and molecules reach a surface where they are absorbed varies with the square root of the rate of flow of solution over the surface. It is suggested that a lethal threshold concentration represents a certain rate at which a poison enters a fish. Thus, an increase in respiratory flow would increase the rate at which the poison reaches the gill surface, and the concentration of poison in solution would then have to be reduced if this rate were to be restored to its former level. It can be shown that the factor X_{sat}/X, which is a measure of the increase in the rate at which poisons reach the gill surface, also varies with the square root of the respiratory flow.

This hypothesis is of some importance, since it implies that any environmental factor that increases the respiratory flow will also increase the rate at which the poison reaches the gill surface, and this may increase the susceptibility of the fish to the poison.

EFFECT OF ACTIVITY

Experiments at this laboratory have shown that increased swimming speed increases the susceptibility of rainbow trout to zinc poisoning (Herbert and Shurben, in prep). This increase in susceptibility is not very great, and, at swimming speeds close to the maximum that trout can sustain for 1 or 2 days, the lethal threshold concentration is only about 0.7 of the lethal threshold concentration obtained in almost still water. Parallel experiments were made to determine the oxygen consumption of these fish at different swimming speeds, and hence to calculate the increase in respiratory flow. The results indicate that the observed increase in toxicity can be accounted for by the increase in respiratory flow, which gives some confirmation to the hypothesis put forward in the previous section.

Schweiger (1957) determined the toxicity of several heavy metals to four species of fish: rainbow trout, char, carp, and tench. In every case the order of sensitivity was trout > char > carp > tench, and he suggested that this order was related to the difference in activity among the species. From data given by Joyner (1961) it appears that the brown bullhead is more resistant to zinc poisoning than are salmonids; it tolerated 12 ppm zinc for 14 days in water of total hardness 74 ppm as $CaCO_3$. He also showed that the rate of uptake of zinc by the brown bullhead (Joyner, cit) is considerably less than the rate of uptake of zinc by salmon fingerlings (Joyner and Eisler, 1961).

TOXICITY OF MIXTURES OF POISONS

MIXTURES OF COPPER AND ZINC

From existing literature, reviewed by Doudoroff and Katz (1953), it appeared that mixtures of zinc and copper salts were much more toxic than would be expected from their individual toxicities. Since such synergism required placing on a quantitative basis, further experiments on the toxicities of mixtures of these two metals were made at this laboratory (Lloyd, 1961 b).

It has been shown in Figure 1 that the log concentration-log survival time curves for copper and zinc salts in both hard and soft waters are identical when the concentrations of heavy metal are expressed as M_s/M_t. If the combined toxic action of these two metals in a mixture were additive, then a similar curve should be obtained if the concentration of the metals is expressed as $Cu_s/Cu_t + Zn_s/Zn_t$. Tests were made with mixtures of zinc and copper salts in a hard water (320 ppm as $CaCO_3$) where $Cu_t = 1.1$ ppm, $Zn_t = 3.5$ ppm, and the ratio $Cu_s/Cu_t : Zn_s/Zn_t$ was 1:2; and in a soft water (15 to 20 ppm as $CaCO_3$) where $Cu_t = 0.044$ ppm, $Zn_t = 0.56$ ppm, and the ratio $Cu_s/Cu_t : Zn_s/Zn_t$ was 2:1. The results are compared in Figure 6 with the curves obtained for the individual metals. It can be seen that a lethal threshold concentration of the mixture in both dilution waters is obtained when the value of $Cu_s/Cu_t + Zn_s/Zn_t$ is equal to unity, but for high concentrations of the mixture in soft water, survival times were considerably shorter than would be expected, indicating that synergism occurs under these conditions. When predicting a safe concentration of a mixture of copper and zinc salts for rainbow trout, however, it may be assumed that the combined action of the two metals is additive.

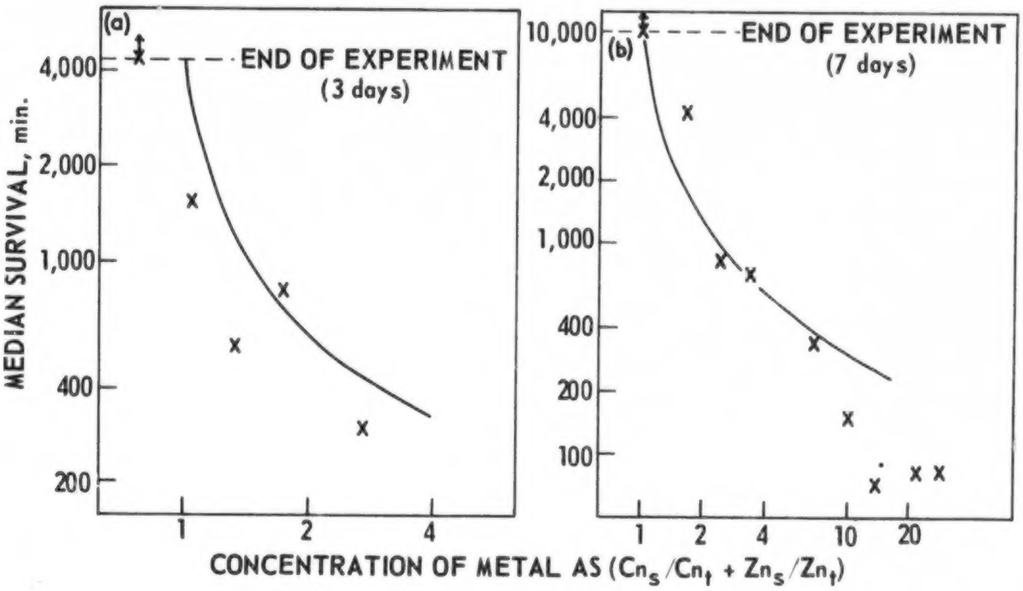

Figure 6. Toxicity of mixtures of copper and zinc sulphate to rainbow trout, (a) in hard water, (b) in soft water. Continuous curves drawn from Figure 1 (a and b).

MIXTURES OF HEAVY METALS WITH OTHER POISONS

Since both copper and zinc exert their toxic effect in a similar way, it is not surprising that the combined effect of low concentrations is additive. These metals may, however, occur in an effluent together with other poisons whose toxic action may be different. It has recently been shown at this laboratory (Herbert, 1961) that a lethal threshold concentration of a mixture of ammonia and zinc salts for rainbow trout is obtained when the value of $A_s/A_t + Zn_s/Zn_t$ becomes unity. The same method can be used to calculate the lethal threshold concentration of a mixture of ammonia and phenol (Herbert, 1962). It is possible, therefore, that the lethal threshold concentration of other pairs of poisons, and also of mixtures containing more than two poisons, might be obtained when the sum of the proportion of the individual lethal threshold concentrations becomes unity.

TOXICITY OF EFFLUENTS CONTAINING HEAVY METALS

Experiments have been made at this laboratory to determine whether the toxicity of actual effluents to rainbow trout could be predicted from their chemical analysis. In one series of tests, Herbert (1962) showed that the toxicity of spent still liquors from gas and coke works could be predicted from their contents of ammonia and monohydric phenols. More recently, work has been extended to sewage effluents, which often contain heavy metals and ammonia as well as a great variety of other toxic substances. Sewage effluents were obtained from several sewage disposal works receiving different proportions and types of trade wastes, and their toxicities were measured under standard conditions (temperature 10° C, pH 7.8 to 8.0, dissolved oxygen 50 percent of the air saturation value). From these tests the 3-day Toxicity Index of the effluents was obtained; this Index is the number of times the effluent has to be diluted to obtain a 3-day TL_m. At the same time, the concentrations of the individual heavy metals ammonia, phenol, and cyanide were determined. From laboratory data on the toxicity of each of these poisons, their individual 3-day TL_m values for the test conditions were calculated; the concentration of each poison found in the effluent was expressed as the proportion of this expected 3-day TL_m, and these values were then summed to give a predicted Toxicity Index. In Figure 7 the relation is shown between the observed and predicted Toxicity Indices for all 12 tests. In two tests there was no mortality in the undiluted effluent (Toxicity Indices < 1), and these were predicted to be nontoxic; in another test there was a 20 percent mortality in the undiluted effluent where the predicted Toxicity Index was 0.9. In the remaining nine tests there was good agreement between the predicted and ob-

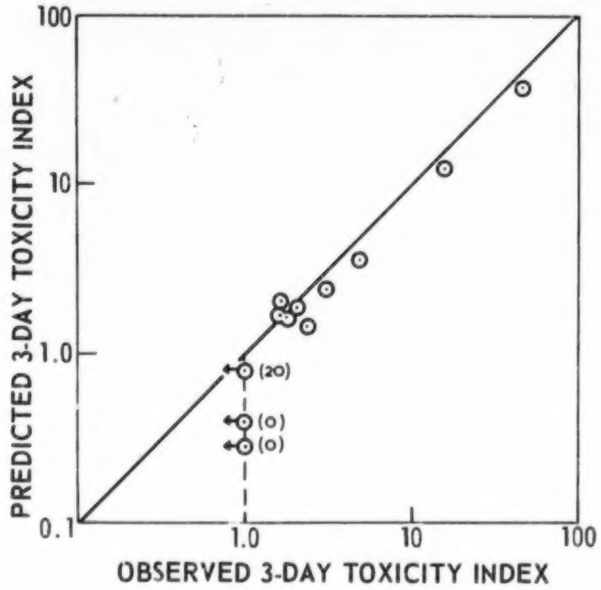

Figure 7. Comparison between predicted and observed 3-day Toxicity Indices for several sewage effluents. Figures in parentheses are percentages of rainbow trout killed in undiluted sewage effluent.

served Toxicity Indices over a wide range of toxicities.

These results are of considerable value, since they indicate not only that the Toxicity Indices of these effluents may be predicted from their chemical analyses, but also that the relative importance of each toxic constituent of the effluent may be assessed. With the sewage effluents used in these tests, the contribution of the heavy metals (chiefly copper and zinc) ranged from 13 to 77 percent of the total toxicity. It is also probable that adequate predictions could be made of the Toxicity Indices of these sewage effluents in dilution waters of different chemical and physical characteristics.

DISCUSSION

The ultimate aim of laboratory bio-assays is to enable one to predict a concentration of poison or effluent that must not be exceeded in a river if fisheries are to remain unharmed. It has long been realized that concentrations found to be safe under strictly controlled laboratory conditions cannot be used unaltered for these predictions, and that an application factor is required to take into account the effects various components of the environment have on the toxicity of the poison, together with a margin of safety. It is becoming clear that the true application factor required will be different for different poisons and for different rivers, and any one standard factor may well be too high in some circumstances and too low in others. The work described in this paper represents an attempt to place on a quantitative basis some of the effects that the chemical and physical nature of the water have on the toxicity of the heavy metals to fish, so that a more accurate estimation of the application factor can be made.

It is fully realized that these experiments go only a little way towards a solution of the problem, since the chemical and physical nature of a river never remains constant but fluctuates, and more field work is required to correlate the results obtained from laboratory investigations with those found in practice. Moreover, the measurement of toxicity used has been the lethal threshold concentration, since this value can be accurately determined, but more information is required about sublethal effects that may result in a gradual deterioration of the condition of the fish. Nevertheless, it is only by a more complete understanding of the basic mechanisms involved in the relation between the susceptibility of fish to poisons and changes in the evironment that any real advance can be made.

ACKNOWLEDGMENT

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SALINITY REQUIREMENTS OF THE FISH, CYPRINODON MACULARIUS*

Otto Kinne †

The basic prerequisites for a sound analysis of the environmental requirements of an organism are (1) long-term rearing and breeding experiments under adequate, ecological conditions in the laboratory, and (2) parallel investigations in the field. It may be useful to assess the environmental requirements of a given group of aquatic life as a whole. More important for understanding of the dynamics of an ecosystem and for proper evaluation of water quality criteria, however, are the requirements of the basic units of the system, i.e., its populations or species. And within each population or species we must know the environmental requirements of the different life cycle stages of individuals, such as egg, larva, and adult, and of the two sexes.

The first step in the analysis of environmental requirements is the establishment of quantitative and qualitative relationships between the organism and its environment. One of the principal difficulties we encounter here is a consequence of two well-known facts: (1) that an organism reacts to its *total* environment rather than to single factors, and (2) that the total environment has to be broken down into single factors in order to facilitate measurement as well as assessment of specific effects and of critical factor intensities. Since it is impossible to measure simultaneously all physical, chemical and biological properties of a natural environment as a whole, a compromise is the only answer - a compromise aiming at a restriction to factors assumed to be most effective.

Such restriction, however, has often been carried too far and numerous papers have been published dealing with one environmental factor only. A monofactorial analysis is dangerous. It may easily lead to wrong conclusions, and should be replaced wherever possible by a bi-, tri- or poly-factorial approach.

Let me exemplify these statements by presenting some of our results concerning the effects of salinity on basic life processes in the euryhaline and eurythermal desert pupfish *Cyprinodon macularius* Baird and Girard. These results demonstrate that other environmental factors, i.e., temperature, and the amount of ambient dissolved oxygen, are capable of considerably modifying or masking the effects of salinity. I shall restrict myself here to some experiments on growth and food conversion in fry and maturing fish, and on rates of embryonic development in eggs. The experiments were conducted under the widely different salinity conditions under which this species is able to exist and reproduce in nature and laboratory. All fish were born and raised under the given conditions.

GROWTH AND FOOD CONVERSION

Growth and food conversion were studied in freshwater and in 15‰, 35‰ and 55‰ (±0.6‰) salinity at various constant temperatures, which were maintained within ± 0.6°C for a 14-hour day and in practically air saturated water (details in Kinne 1960).

The growth curves obtained in the four salinities and at a constant temperature of 30°C are shown in Figure 1. At 30°C, growth rates decrease in the following order: 35‰, 15‰, 55‰, freshwater. This order is subject to considerable change in different temperatures. A comparison between fish raised in freshwater and others raised in 35‰ salinity (Figure 2) shows that at the lower temperature levels, 15° and

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[*] Professor Meyer-Waarden to his 60th birthday.

[†] Biologische Anstalt Helgoland, Hamburg-Altona, Germany

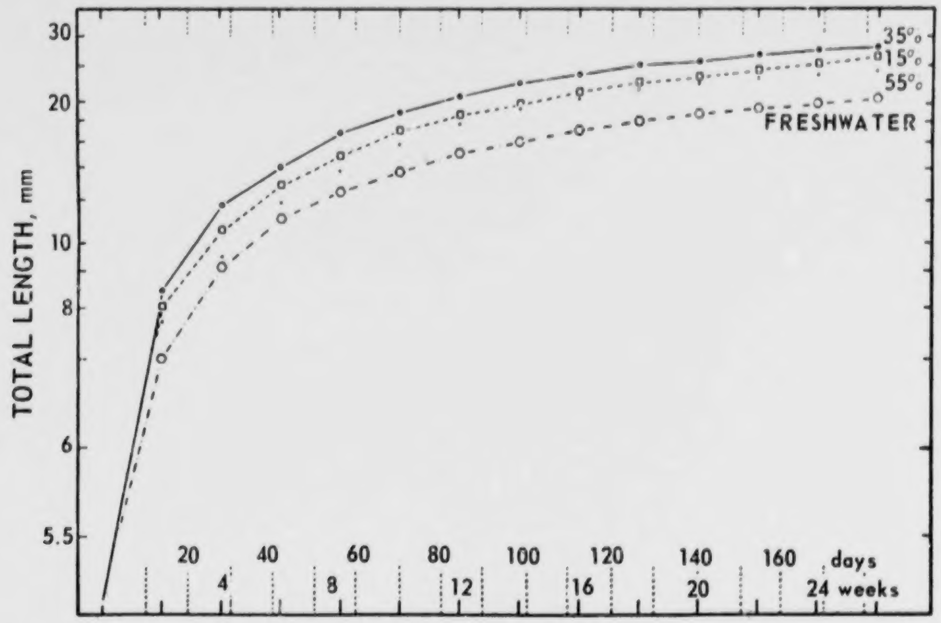

Figure 1. Growth rates of *Cyprinodon macularius* reared in four different salinities at 30°C. Semilogarithmic plot. Each value represents the average total length of 12 individuals. Horizontal: age in days and weeks. All fish were raised under the given conditions (From Kinne 1960).

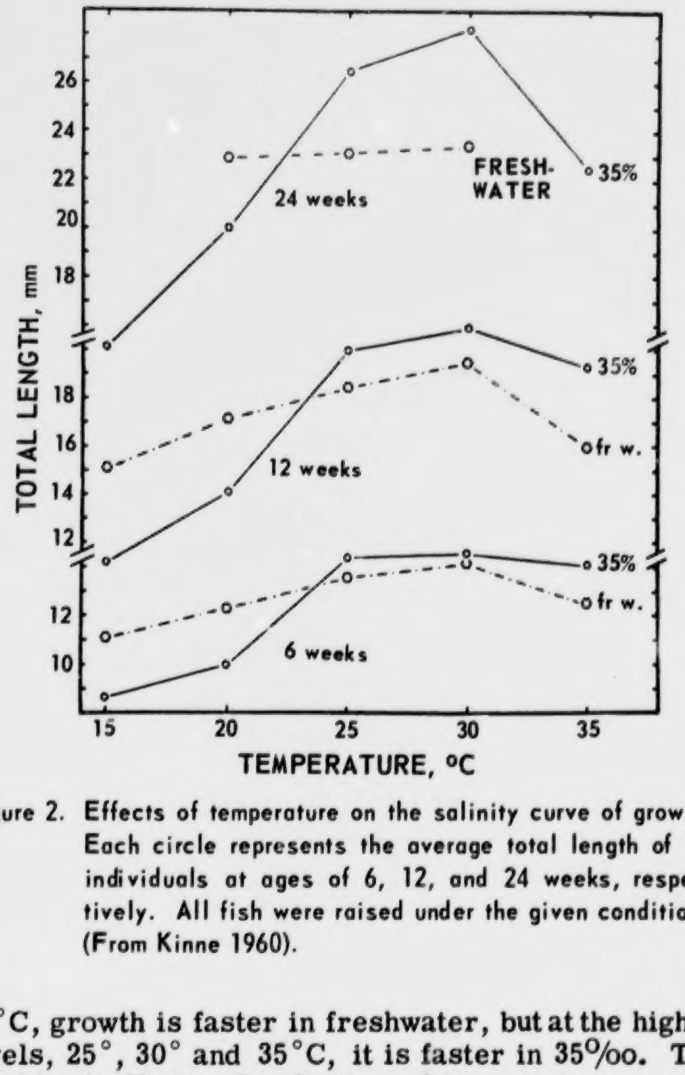

Figure 2. Effects of temperature on the salinity curve of growth. Each circle represents the average total length of 12 individuals at ages of 6, 12, and 24 weeks, respectively. All fish were raised under the given conditions (From Kinne 1960).

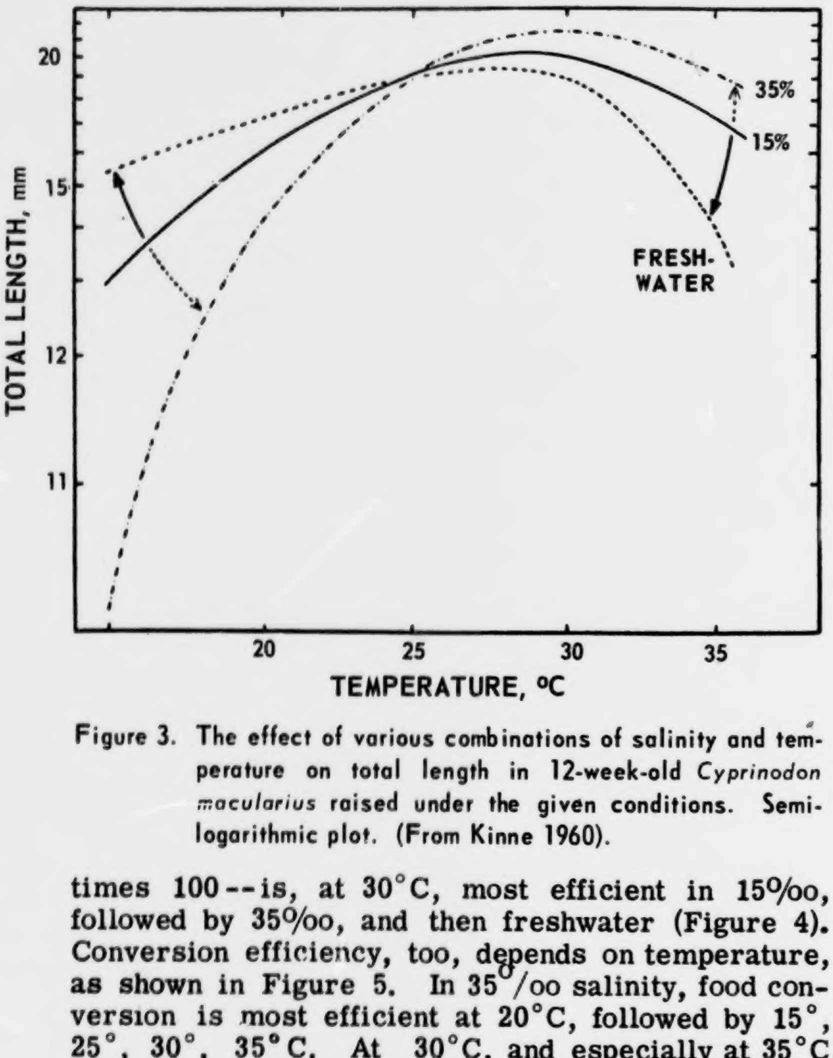

Figure 3. The effect of various combinations of salinity and temperature on total length in 12-week-old *Cyprinodon macularius* raised under the given conditions. Semilogarithmic plot. (From Kinne 1960).

20°C, growth is faster in freshwater, but at the higher levels, 25°, 30° and 35°C, it is faster in 35‰. The combined effects of salinity and temperature are illustrated diagrammatically in Figure 3; the temperature curve of growth established for fish born and raised in 15‰ salinity rotates clockwise in lower salinities, but counterclockwise in higher salinities, the curves intercept at approximately 25°C.

Food conversion--i.e., increase in dry weight of fish, divided by dry weight of white worms eaten, times 100--is, at 30°C, most efficient in 15‰, followed by 35‰, and then freshwater (Figure 4). Conversion efficiency, too, depends on temperature, as shown in Figure 5. In 35°/oo salinity, food conversion is most efficient at 20°C, followed by 15°, 25°, 30°, 35°C. At 30°C, and especially at 35°C, the fish defecated at a tremendous rate; at 35°C, they produced whitish feces, representing worms that had passed the intestinal tract almost untouched by digestive processes. The efficiency of food conversion depends also on other factors, especially on food supply and age (Kinne 1960). At 30°C and under restricted food supply conditions, the average efficiency over a period of 16 weeks was found to be 14.4 in 15‰, 10.6 in 35‰, and 8.8. in freshwater;

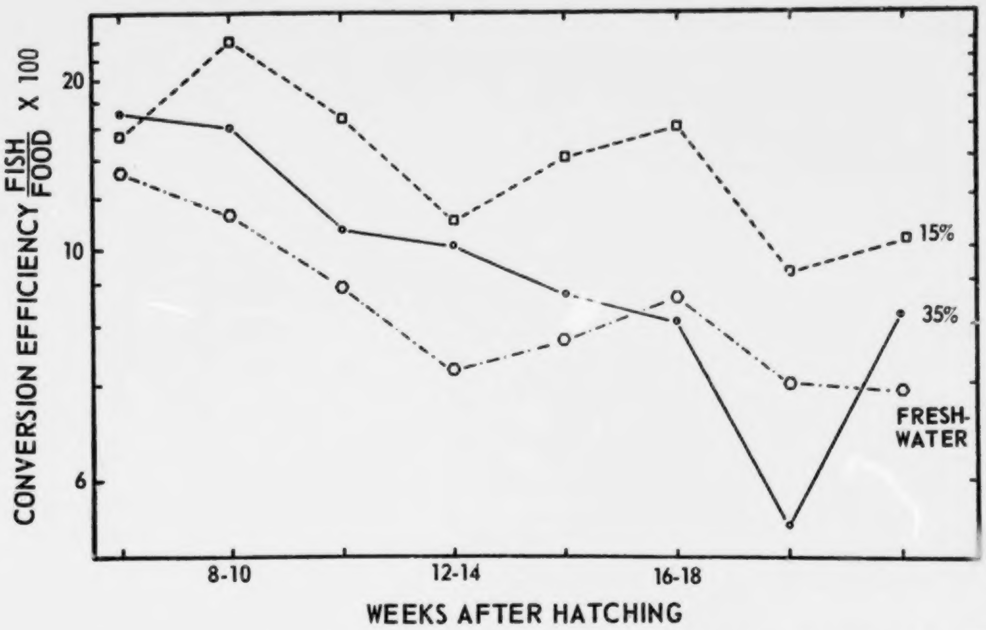

Figure 4. Efficiency of food conversion in *Cyprinodon macularius* raised in three different salinity levels at 30°C. Semilogarithmic plot. Each value represents the average conversion efficiency per fish per 2 weeks and is based on 12 individuals kept under conditions of restricted food supply. (From Kinne 1960).

corresponding values obtained under conditions of unrestricted food supply were 1.3 in 35‰ and 0.9 in freshwater. (Except for these data, all efficiencies quoted refer to results obtained under conditions of restricted food supply; details in Kinne 1960). Conversion becomes significantly less efficient with increasing age.

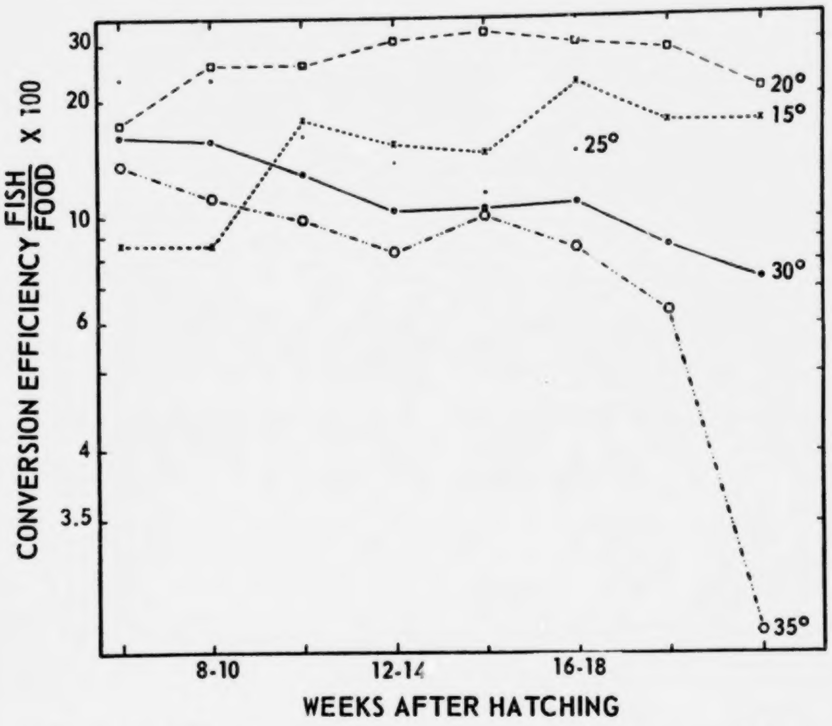

Figure 5. Efficiency of food conversion in *Cyprinodon macularius* raised at five different temperature levels in 35°/oo salinity. Semilogarithmic plot. Each value represents the average conversion efficiency per fish per 2 weeks and is based on 12 individuals kept under conditions of restricted food supply. (From Kinne 1960).

EMBRYONIC DEVELOPMENT

Eggs were laid and fertilized in a salinity of 35 ± 2‰ at water temperatures between 26° and 28°C, and in practically air-saturated water (14-hour day). They were transferred into incubators approximately 4 hours after spawning and incubated in five different salinities: 1/2 freshwater (one part glass distilled water plus one part tap water), 35‰, 45°/oo, 55‰ and 70‰ (details in Kinne and Kinne 1962). Under these conditions, the criteria used to determine the rate of embryonic development were the length of time between fertilization and hatching, and the length of time required by the embryo to reach certain embryonic stages. The application of these two methods was necessary in order to be able to dis-

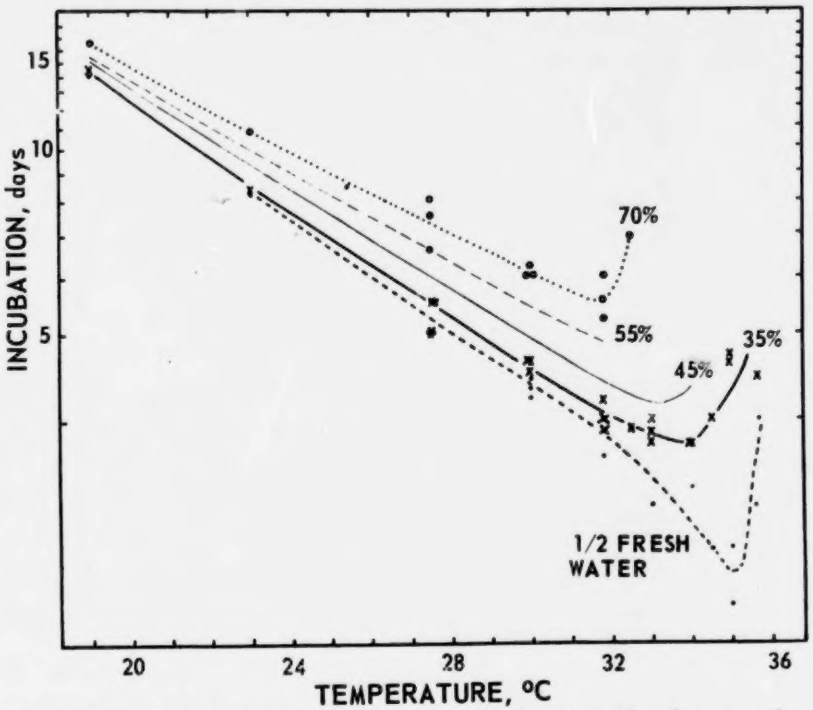

Figure 6. Incubation periods in days as function of salinity and temperature in eggs of *Cyprinodon macularius*. Semilogarithmic plot; handfitted curves; individual data for 45°/oo and 55°/oo omitted. Average data from 10 to 15 eggs in each case. Salinities remained practically constant in 1/2 freshwater, they varied up to 2°/oo between 35°/oo and 45°/oo, and up to 3.5°/oo between 45°/oo and 70°/oo. Water temperatures were maintained within ± 0.2°C up to 30oC and within ± 0.1°C between 30°C and 36°C. (From Kinne and Kinne 1962).

criminate between effects on the development proper and on the process of hatching. In most cases, the two methods produced similar results.

Figure 6 shows in a semilogarithmic plot the incubation periods for eggs incubated in the 5 different salinities at various constant temperatures between 19°C and 36°C and at oxygen levels equivalent to 100 ± 5 percent air saturation. It reveals a progressive retardation of developmental rate with increasing salinity. This retardation is significantly affected by temperature; the differences in the five salinity levels are progressively augmented as the temperature increases. Towards higher temperatures, the curves show inflections indicating the position of the respective upper critical temperatures (in 55‰ no data were available above 31.8°C). The inflections occur approximately: in 1/2 freshwater at 35°C, in 35‰ at 34°C, in 45‰ at 33.3°C, and in 70‰ at 31.8°C. The upper lethal temperature of incubation (100% mortality), too, is a function of salinity; in 1/2 freshwater and in 35‰ it lies near 36°C, in 45‰ near 34.2°C, and in 70‰ near 32.5°C.

These effects of salinity on rates of embryonic development, and on the upper critical and lethal temperatures are statistically highly significant and easily reproducible. Nevertheless, a further analysis discloses that it is not the salinity per se that causes these effects but the concomitant change in the level of saturation for ambient dissolved oxygen. Figure 7 shows that the saturation values of oxygen from normal dry atmosphere in water of different salinities vary from 6.7 milliliters of oxygen per liter (1/2 freshwater at 19°C) to 3.2 milliliters of oxygen per liter (70‰ at 36°C). This represents a total reduction of 52 percent.

A series of experiments conducted at oxygen levels equivalent to 70 ± 10 percent air saturation and to 300 ± 80 percent air saturation demonstrated the importance of the amount of oxygen available to the embryo (details in Kinne and Kinne 1962).

A comparison between values obtained in 70 percent air-saturated water and in 100 percent air-saturated water (Figure 8), reveals that development proceeds at similar rates up to a temperature of 15° to 18°C; at higher temperatures, the incubation period becomes increasingly longer in water of 70 percent air saturation. In 35‰ and 27.5°C, incubation periods are about 25 percent longer than under conditions of 100 percent air saturation. The upper lethal temperature of incubation drops in 1/2 freshwater to approximately 28°C and in 35‰ to approximately 27.5°C.

In 300 percent air-saturated water, development is significantly accelerated in 35‰ and 70‰ (Table 1). At 32°C development is accelerated 12 percent in 35‰ and 27 percent in 70‰, relative to the respective results obtained in water of 100 percent air saturation. There is practically no acceleration in 1/2 freshwater. At 34°C, incubation periods are shorter in 35‰ than in 1/2 freshwater at 100 percent air saturation, and rates are almost the same in 70‰ salinity as 1/2 freshwater at 100 percent air saturation. The upper lethal temperature of incubation shifts upward, and lies, for example, in 70‰ at approximately 34.5°C (it lies near 32.5°C in 100 percent air saturation). These and other results indicate that the retardation effect observed in high salinities can be nullified largely or completely by a rise in the amount of dissolved oxygen. In fact, salinity per se, that is, salinity as osmotic stress, seems to exert little influence.

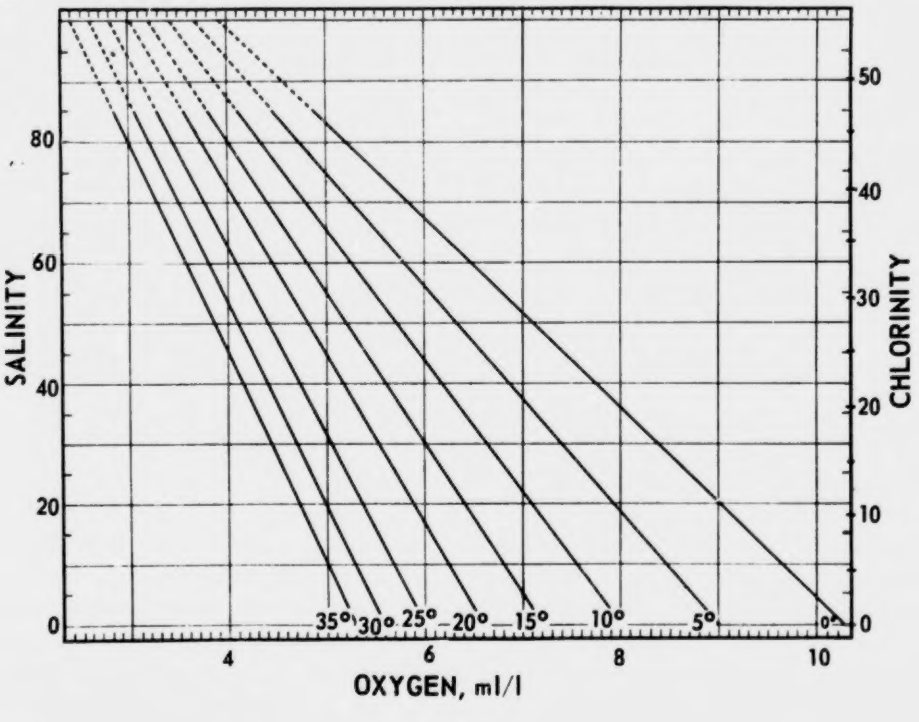

Figure 7. Saturation values of oxygen (ml/l) from normal dry atmosphere at 760 mm, for salt concentrations ranging from glass-distilled water to 85‰ salinity at eight different temperatures. (From Kinne and Kinne 1962).

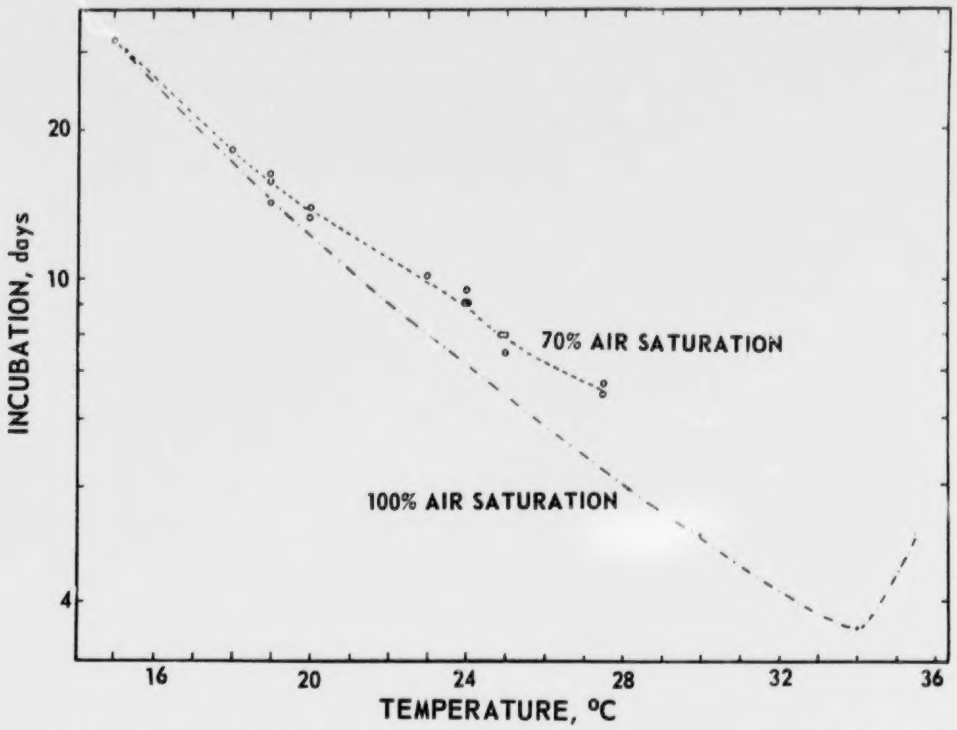

Figure 8. Incubation periods in days as function of the amount of ambient dissolved oxygen available to the eggs in 35°/oo salinity at various constant temperatures. Circles represent average data from 10 to 15 eggs in 70% air saturation; hand-fitted curve. 100%-air-saturation-curve from Figure 1. Semilogarithmic plot. Control of salinity and temperature as in text to Figure 1. (From Kinne and Kinne 1962).

Table 1. INCUBATION PERIODS IN DAYS IN DIFFERENT COMBINATIONS OF TEMPERATURE, SALINITY, AND OXYGEN. EACH EXPERIMENT WAS CARRIED OUT WITH 15 EGGS. (From Kinne and Kinne 1962).

°C	Half freshwater		35°/oo S		70°/oo S	
	100% * Air saturation	300% Air saturation	100% Air saturation	300% Air saturation	100% Air saturation	300% Air saturation
32.0	3.85	3.9	4.1	3.6	5.5	4.0
33.0	3.5	3.5	3.85	3.3	lethal	3.8
34.0	3.4	3.5	3.8	3.2	lethal	3.5
35.0	3.25	3.2	4.5	3.6	lethal	lethal

*Approximate saturation, giving the amount of dissolved oxygen present as percentage of the air saturation value at a given temperature and salinity and an atmospheric pressure of approximately 760 mm.

Comparable effects of reduced levels of dissolved oxygen on embryonic development in fishes have been reported by a number of authors. Johansen and Krogh (1914) were among the first biologists to expose developing fish embryos to different levels of dissolved oxygen. They reported that levels below 50 percent air saturation cause delay in the development of plaice incubated at 6° to 7°C. Similar retarding effects have since been described for a variety of fish species by Stockard (1921), Lindroth (1942, 1946), Einsele (1956), Seymour (1956,1959), Alderdice et al. (1958), and Garside (1959, 1960). Lindroth (1942, 1946) working on the Atlantic salmon, *Salmo salar*, and the pike, *Esox lucius*, found that the retarding effects of reduced levels of dissolved oxygen increase at higher temperatures, and the same relation was later reported by Garside in *Salvelinus namaycush* (in 1959), *Salvelinus fontinalis*, and *Salmo gairdneri* (in 1960). Our results on *Cyprinodon macularius* are in full agreement with these findings.

Growth, food conversion, and embryonic development are basic life processes. In *Cyprinodon macularius* they are significantly affected by salinity. I hope I have demonstrated that the specific effects of salinity on these life processes and the salinity requirements of this fish are intimately correlated to other environmental factors, and that the importance of the salinity factor as part of the total environment of *Cyprinodon macularius* can only be fully appreciated and understood within the framework of a comprehensive, poly-factorial analysis.

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DISCUSSION

DISSOLVED OXYGEN REQUIREMENTS OF FISHES

Q. Do differences in size of fish at hatching persist if the sac fry are maintained under the conditions of reduced oxygen?

A. Some of our early results indicated that size differences do persist after hatching but later experiments indicated that the differences may be small.

Q. Do water velocities of 6 centimeters per hour over the eggs have a different effect from a velocity of zero?

A. We haven't tried zero so I cannot answer your question except to say that any increase above 3 centimeters per hour produced an effect.

Q. In the swimming experiments, are the fish able to swim in low dissolved oxygen simply because they are incurring an oxygen debt? When we discuss the avoidance response of fish to low dissolved oxygen, we must be careful to avoid teleological explanations.

A. We measure the sustained swimming speed and within limits this speed is not affected by the interval of time between water velocity increases. Therefore, this is probably a test of fatigue and not of the ability to incur an oxygen debt. Early work was done with apparatus that could not exhaust fish rapidly because the water velocity could not be increased enough. In that work, fish swam as long as 48 hours before exhaustion. This indicates failure is not due to sudden stress and, therefore, not due to an oxygen debt. In regard to your comment about teleological explanations, you are correct, but our data do indicate a directed response and not just random movements.

Q. Is the variation in dissolved oxygen found in natural waters the reason that growth is less there than under more uniform laboratory conditions?

A. Fluctuations in dissolved oxygen have been shown to be detrimental to growth; growth is similar to growth that would be obtained at a constant dissolved oxygen level equal to the lowest of the fluctuating concentrations.

Comment - This lesser growth may also be due to the inaccessibility of food. For example, we took smallmouth bass from a stream and planted them in a pond and the pond population grew better.

Comment - The Ohio River Sanitation Commission standards for dissolved oxygen stipulate that dissolved oxygen shall not go below 5 ppm for periods exceeding 8 hours and never below 3 ppm. These times are not rigid or exact and the concentrations are tentative. We feel that tentative criteria are necessary, however, until better information is available.

Comment - At the time these standards were set they seemed to provide adequate safety, but they are hard to enforce and it now appears that a single minimum concentration might be better.

THE ENVIRONMENTAL REQUIREMENTS OF CENTRARCHIDS WITH SPECIAL REFERENCE TO LARGEMOUTH BASS, SMALLMOUTH BASS, AND SPOTTED BASS

Q. Is an increase in weight of 150 or 170 percent in 15 days an unusual growth rate for bass 80 to 90 mm long?

A. I think such an increase is accomplished in the natural habitat. We consider a growth to one-half pound in 3 months not unusual.

WATER QUALITY CRITERIA FOR FISH LIFE

Q. Is 5 ppm dissolved oxygen a maximum or an average concentration desirable for salmonid waters or is it a concentration necessary at a certain time of day?

A. Lower concentrations can be accepted locally and temporarily.

Q. Is the level of 5 ppm dissolved oxygen based on tolerance or some other consideration?

A. It is based on normal growth and development.

TOXICITY OF SOME HERBICIDES, INSECTICIDES, AND INDUSTRIAL WASTES

Q. In the experiments with sodium chloride, was there a relationship between size and length of the fish and susceptibility? Some workers have found a closer relationship to size than to species of salmonids.

A. We used only one size of fish.

Q. What type of water was used in these experiments? I would expect different and variable results depending on the hardness of the water.

A. This work was done in a hard rather alkaline water.

Comment - This may explain the high tolerance you found.

Q. In long-term tests with sodium chloride, did the fish shed their scales?

A. I think so.

THE TAINTING OF FISH BY OUTBOARD MOTOR EXHAUST WASTES AS RELATED TO GAS AND OIL CONSUMPTION

Q. Could the outbreak of *Columnaris* have been affected by pollution; in this case, outboard motor exhaust wastes?

A. This possibility did not occur to us.

Q. Did panel members know when they were getting fish from a contaminated pond?

A. The members did not know what type of fish they were tasting.

Q. What would be the effects of circulation and size of the body of water?

A. This lake was stratified with a thermocline at 15 to 20 feet.

Q. What about the levels of lead in the water?

A. Lead was less than 10 micrograms per liter, which is the lowest level we could detect.

Comment - The 96-hour TL_m for outboard motor wastes was reached at a fuel consumption of 1 gallon per 1900 gallons of water.

Q. How long do fuels remain in suspension and what would usage of these lakes in previous years have on present levels?

A. Evidence from carbon column extracts indicated the carryover from previous years was below detectable levels and persistence would be a matter of weeks.

EFFECTS OF OIL POLLUTION ON MIGRATORY BIRDS

Q. Do you know of any references to oil pollution on the Great Lakes themselves?

A. One study was made on the Great Lakes and they were found to be generally clean. There have not been many studies made on the loss of wildlife due to oil pollution.

FACTORS WHICH AFFECT THE TOLERANCE OF FISH TO HEAVY METAL POISONING

Q. Would someone venture an opinion as to whether the antagonistic effect of calcium on the toxicity of heavy metals might be due to a loose combination of calcium ion with the mucous protein thereby making such binding sites unavailable to the metal ion?

A. (Hiltibran, Ill. Nat. Hist. Survey) In our laboratory we investigated the effect of several divalent metal ions on the oxidative phosphorylation systems of bluegill liver mitochondria, and found that zinc, cadmium, calcium, and manganese ions inhibit oxygen uptake. But when we investigated the adenosine triphosphatase systems of mitochondria, we found most enzyme activity in the presence of zinc and cadmium ions. Calcium and manganese ions were less effective. Magnesium enhances oxygen uptake; it gave about the same order or magnitude of results in the adenosine triphosphatase systems as did calcium and manganese. The two enzyme systems I have mentioned are part of the over-all oxidative phosphorylating sequence of enzymes by which energy is produced and converted into usable compounds; however, in one part of the sequence zinc, cadmium, calcium, and manganese ions were inhibitory, but in another part of the over-all system they promoted enzyme activity. Thus it would appear that there is a very delicate balance of metal ions within cells, and if the concentration of some ion is increased, then this metal ion could alter some part of the physiological processes. If this inhibition becomes severe, death might result.

In the case of the effects of metals on protein, I believe the factors you may be observing are the various gross physiological responses, which are based or controlled by underlying biochemical processes. When these latter processes are altered or inhibited then all other additional physiological processes that are based upon the underlying chemical reactions also stop and the fish dies. For example, if energy production is blocked at some point and energy production ceases, then all physiological responses requiring energy also stop. Many other systems may be affected in a similar manner.

We have conducted some experiments to determine the competition between metal ions on oxygen uptake. We found that magnesium ions can overcome the inhibition of oxygen uptake by manganese ions, but magnesium ions apparently do not alter the effect of manganese ions on the phosphorylating reactions. We have not pursued this area of research to any extent.

We have not investigated the effect of copper ions so I cannot make any comment as to their effect.

Calcium is an oxidative phosphorylation uncoupler; that is, it interferes with the transfer of energy from oxidation of substrates to organic phosphate compounds. In the bluegill liver mitochondria systems calcium does not appear to act as an oxidative phosphorylation uncoupler due to inhibition of oxygen uptake. Usually uncouplers do not inhibit oxygen uptake.

Many divalent metal ions *in vitro* are protein precipitants. It is possible that relatively high concentrations of metal ions within cells might cause some disarrangements of the proteins of the cell, which also could cause loss of function.

In another session there was some mention of selenium, both as a toxic principle and in its physiological role. I would like to mention that vitamin B_{12}, an important physiological compound, contains a cobalt ion and a cyano group as part of its structure; this is another case where the physiological compound contains elements or groups that under other conditions might be extremely toxic.

Thus we are concerned with a very complex situation, which is going to require much work to unravel.

Q. Would the effects of two toxicants with dissimilar modes of action be additive, for example, arsenic and copper?

A. I cannot say except to point out that zinc and ammonia are additive although the mode of action is different.

Q. Have you noted a difference in gill histology among fish maintained in water with different calcium contents? We have noticed that fish kept in hard water have more mucus on the gills, which could suggest the role of calcium.

A. I have not noticed a difference, but I have not really looked for one.

SALINITY REQUIREMENTS OF THE FISH CYPRINODON MACULARIUS

Q. Is there a difference in activity in the two extremes of salinity?

A. We found that temperature, but not salinity, produced significant differences in activity.

THE CONCENTRATION OF RADIONUCLIDES IN AQUATIC ORGANISMS, THEIR PASSAGE THROUGH THE FOOD CHAIN, AND POSSIBLE EFFECTS

Conrad P. Straub[*], *Chairman*

RADIOACTIVITY IN FRESHWATER ORGANISMS OF SOME LAKES OF NORTHERN ITALY [†]

Oscar Ravera [‡]

The purpose of this study was to compare the existing radioactivity (natural radioactivity plus fall-out) in the various levels of the lacustrine food-chain.

The lakes investigated during the last 2 years (1960-1961) are presented in Figure 1. Figure 2 shows the collection zones of Lake Maggiore, where most of this research was carried out. Three characteristic cross-sections of the lake are also given: A-A' for the northern basin, which has a maximum depth of 372 meters; B-B', for the basin controlled by the Toce River; and C-C', for the southern basin, both of which have a maximum depth of 100 meters.

Total beta activity was measured with an equipment for anticoincidence counting with two Geiger-Müller tubes with a 1" window. A 100-channel scintillation apparatus was used for gamma spectrometry. Mean values of total beta activity calculated for the material collected in the three basins of Lake Maggiore are listed in Table 1.

The radioactivity of plankton and fishes was studied by A. Berg and the data on this subject listed in Table 1 are reported in Chapter VII of the Final Report of IAEA Contract No. 59 (1961).

The radioactivity of phytoplankton was higher than that of zooplankton. This is probably due to the higher ratio of surface to volume in algal cells as compared with zooplankton. The radioactivity of the whole body of fish was influenced very little by season or zone of collection whereas it was greatly affected by the age of the fish. Slight differences in radioactivity were noted among the three species studied (*Coregonus* sp., *Alburnus alborella*, De Fil. and Lota lota, L.); these differences are probably the result of differences in diet.

Benthos collected in different zones, seasons, and depths had a total beta activity of about 1 picocurie per gram wet weight.

No significant differences were noted for sediments collected in different basins but at the same depth.

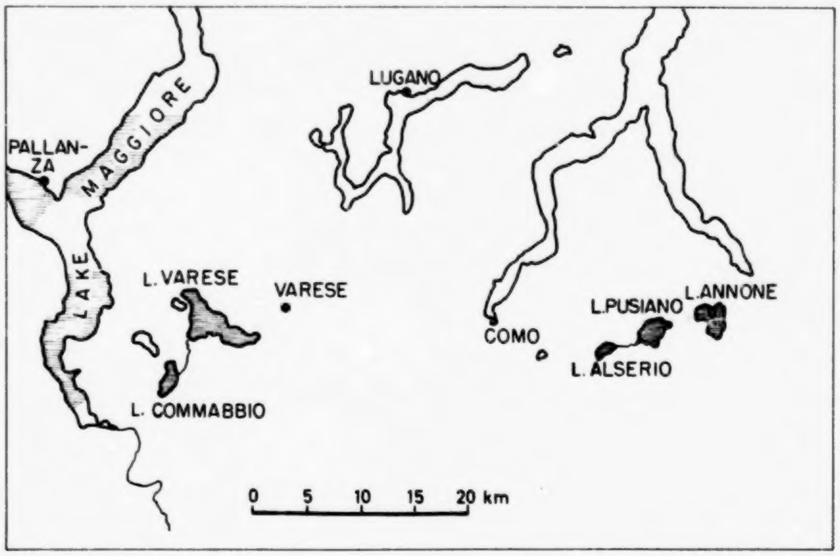

Figure 1. Lakes from which the samples have been collected: L. Maggiore, L. Alserio, L. Pusiano, L. Commabbio, L. Annone and L. Varese.

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[*] Chief, Radiological Health Research Activities, Robert A. Taft Sanitary Engineering Center.

[†] A great part of this work was carried out for the IAEA Contract No. 59 (1961): Berg, A., Margaret Merlini, O. Ravera and V. Tonolli; Accumulation of Fission Products from Fall-out in Lake Biota (Lago Maggiore).

[‡] Biologia, EURATOM, Ispra, Italy.

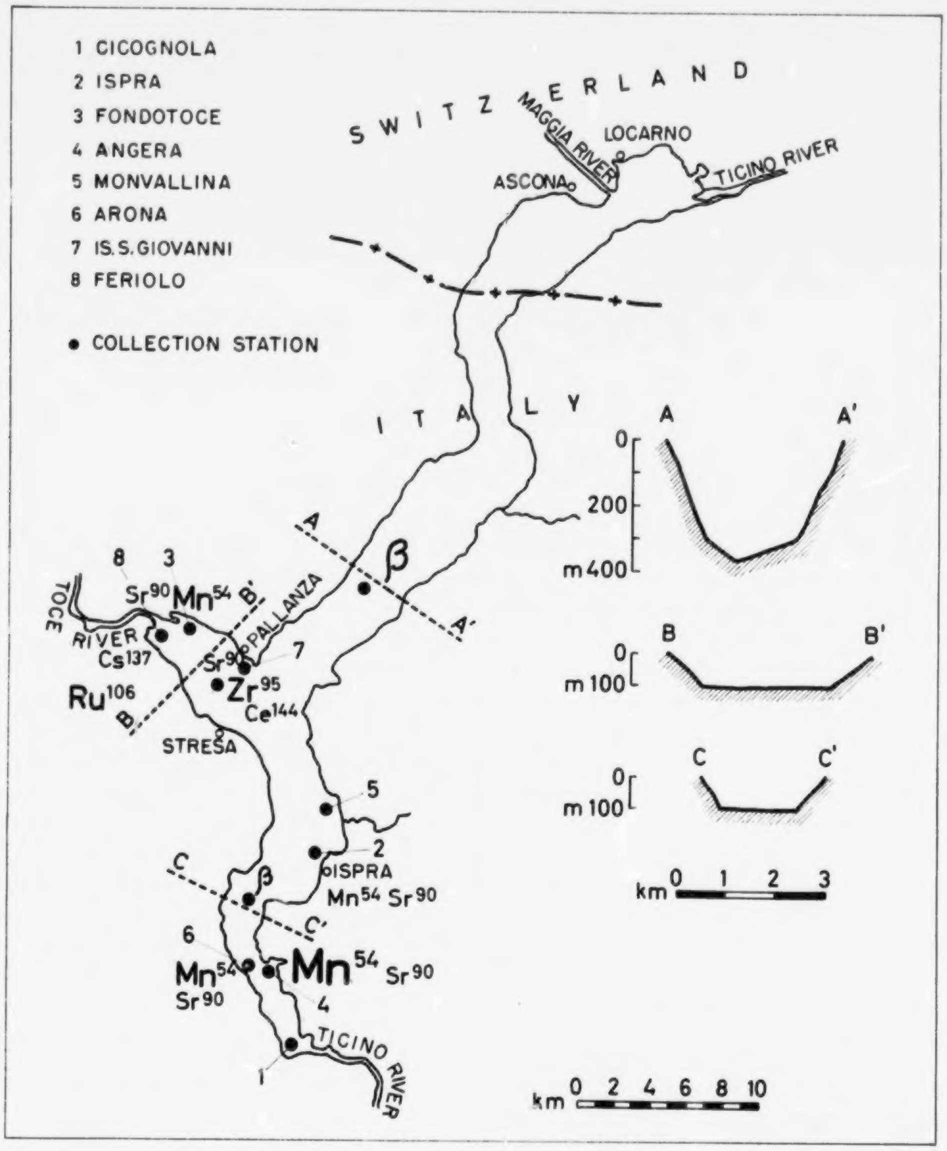
1 CICOGNOLA
2 ISPRA
3 FONDOTOCE
4 ANGERA
5 MONVALLINA
6 ARONA
7 IS.S.GIOVANNI
8 FERIOLO
● COLLECTION STATION
SWITZERLAND
MAGGIA RIVER
LOCARNO
ASCONA
TICINO RIVER
ITALY
TOCE RIVER
PALLANZA
STRESA
ISPRA
TICINO RIVER
Sr^{90} Mn^{54}
Cs^{137}
Ru^{106}
Zr^{95}
Ce^{144}
β
Mn^{54} Sr^{90}
A A'
B B'
C C'
0 200 m400
0 m100
km 0 1 2 3
km 0 2 4 6 8 10

Figure 2. Lake Maggiore-stations where the samples were collected.

Table 1. MEAN TOTAL BETA ACTIVITY IN SAMPLES OF FRESHWATER ORGANISMS FROM LAKE MAGGIORE

Sample			Mean values (pc/g wet wt.) of the total beta activity		
			Northern basin	Toce River basin	Southern basin
Plankton	zoo-		1.0	---	1.0
	phyto-		1.2	---	1.2
Fishes	*Coregonus*		2.5	---	---
	Alburnus		2.2	---	2.3
	Lota		—	1.4	1.6
Larger aquatic plants			---	2.2	1.6
Periphyton			---	8.4	1.2
Benthos			1.0	1.2	1.7
Sediments			25.7	---	26.9
Molluscs	*Viviparus*	Shell	---	5.6	3.6
		Soft tissues	---	1.2	1.1
	Unio	Shell	---	---	2.0
		Soft tissues	---	---	2.8

As far as the larger aquatic plants are concerned individuals of the same species, e.g. *Lagarosiphon*, growing in different places had different values of radioactivity. Interspecific differences were found within the same zone. The beta- and gamma-activity of periphyton was higher than that of its substrate (Figure 3).

The Molluscs are very important organisms in the littoral biocoenosis because of their biomass and their wide distribution. *Lymnaea peregra*, L., and *Physa acuta* (Gastropoda, Pulmonata), *Viviparus ater*, Cris. and Jan (Gastropoda, Prosobranchia) and *Unio mancus* var. *elongatulus* (Lamellibranchia) were the species studied, but a great part of the work was carried out on the latter two species.

The data in Table 1 show that the radioactivity calculated for the soft tissues of *Unio* was higher than that measured for the shell, whereas in *Viviparus* the radioactivity of the shell was higher than that of the soft tissues; also, *Viviparus* shell showed a higher radioactivity than *Unio* shell.

Samples were collected from seven stations to evaluate the radioactivity differences in the shell and in the soft tissues in *Viviparus* of either sex dwelling in different zones. Each sample, composed of at least 100 individuals collected at random, was representative of the whole population in a zone. The data in Table 2 lead to the following conclusions: 1) an increase in the radioactivity of the shell corresponds to an increase in radioactivity of the soft tissues, 2) great differences of shell radioactivity appear between stations and between sexes, and 3) small differences in radioactivity of the soft tissues are observed between populations.

Table 2. TOTAL BETA ACTIVITY (pc/g wet wt.) OF *VIVIPARUS ATER* COLLECTED IN SEVEN STATIONS OF LAKE MAGGIORE

Station	Total beta activity			
	Shell		Soft tissues	
	Male	Female	Male	Female
Cicognola	1.2	3.6	0.6	0.6
Ispra	2.8	3.0	1.0	0.7
Fondotoce	3.5	3.5	1.0	0.8
Angera	3.7	5.6	1.1	1.0
Monvallina	3.9	4.4	1.5	1.3
Arona	4.6	3.5	1.6	1.6
Is. S. Giovanni	9.6	5.7	2.0	1.4

Because the concentration of strontium-90 in the shell of *Viviparus* differed very little among populations and between sexes, the total radioactivity of the shell can be explained only in part by its strontium-90 content (Table 3); it is therefore necessary to assume the presence of one or more beta emitters not yet

Total beta-activity (pc/g w.w.)

Station	Lagarosyphon	Periphyton
Feriolo	2,15	8,37
Is. S. Giovanni	1,94	3,83
Cicognola	1,59	6,40
Ispra	1,42	6,24

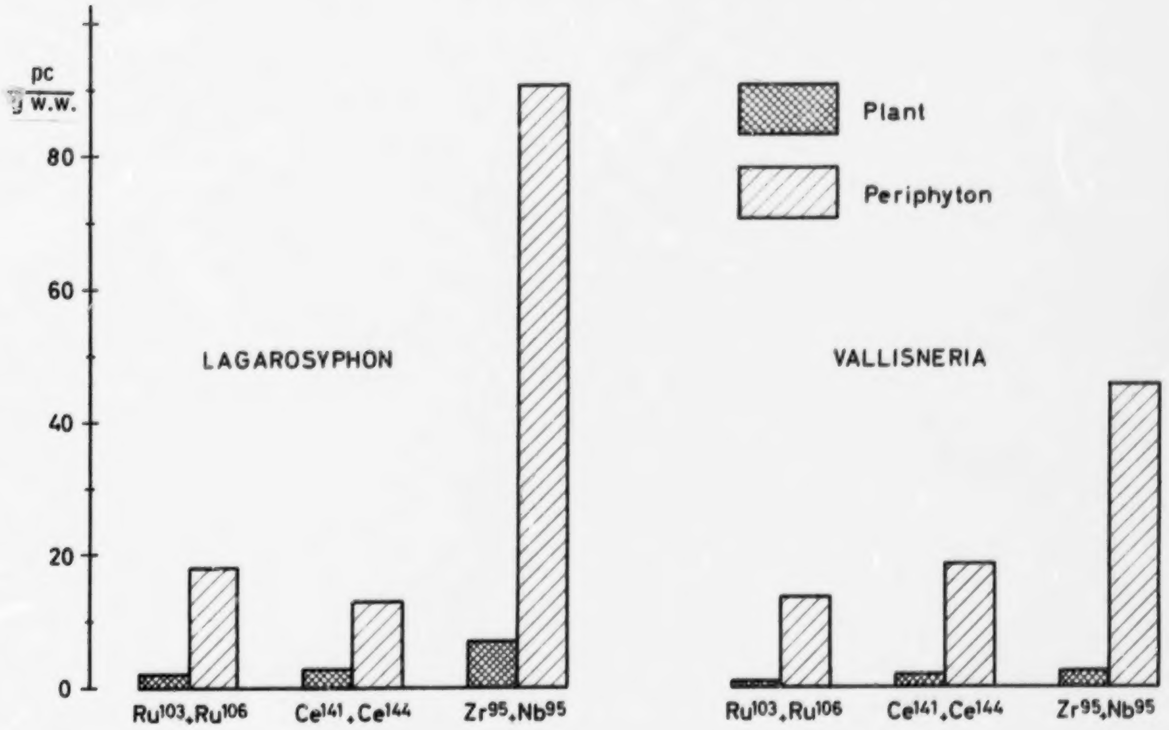

Figure 3. Concentration of the radionuclides in the aquatic plants and periphyton.

identified. These beta emitters probably are not present in the shell of *Unio* except in traces because in this material the strontium-90 concentration reached a value slightly lower than that of the total beta activity.

The higher level of radioactivity reached in the soft tissues of *Unio* (in comparison with that of the soft tissues of *Viviparus)* was probably due to the fact that the tissues of *Unio* always contained a certain amount of manganese-54, whereas this radionuclide was never found in *Viviparus* (Figure 4). Using the same method, we were unable to detect manganese-54 in lake water, fishes, aquatic plants, or sediment (Ravera and Vido, 1961), which indicates that the concentration of this radionuclide in these materials was lower than the sensitivity of the instruments used.

The importance of the role played by age on the concentration of strontium-90 and manganese-54 in *Unio* was evaluated. Figure 5 clearly shows the relation between the manganese-54 and strontium-90 contents and the age of the organism. The concentration of strontium-90 decreased with increasing age, whereas the opposite was true for manganese-54. This different pattern may be explained by the following facts: 1) *Unio* may live more than 10 years, (Pelseneer, 1935); 2) the metabolic rate of the shell is very slow in comparison with that of the soft tissues; 3) strontium-90 and manganese-54 are artificial radionuclides that have diffused in the biosphere only in recent years.

Table 3. STRONTIUM-90 CONCENTRATION IN SHELLS OF *VIVIPARUS ATER* COLLECTED IN DIFFERENT ZONES OF LAKE MAGGIORE

Station	Sr^{90} in Shell (pc/g wet wt.)	
	Male	Female
Ispra	1.3	1.6
Monvallina	1.4	1.5
Is. S. Giovanni	1.7	1.7
Arona	1.9	1.6

Therefore, the oldest individuals secreted the greater part of their shell in an environment without radiostrontium, whereas the youngest ones secreted all, or a great part, of their shell when the lake was contaminated by this radionuclide. The higher concentration of manganese-54 in the oldest individuals is presumed to be due to the accumulation of this nuclide in the body of *Unio* during its life-span. Because the samples of *Unio* collected at four stations were very large it seemed improbable that the age of the molluscs could be considered responsible for the different manganese-54 content calculated for the various populations. Therefore this pattern is probably due to the different manganese concentrations at the four stations (Table 4).

The data in Table 5 show the differences in radioactivity among different parts of the body of *Unio* and *Viviparus*.

Because the sediment, which plays a very important role in the nutrients turnover of a lake, had a very high radioactivity, it seemed interesting to compare the values for the sediments collected at different depths. Figure 6 shows that the mean total beta activity increases with depth, from less than 1 meter to 50 meters, whereas from 50 meters to 360 meters it remains constant at 31 picocuries per gram wet

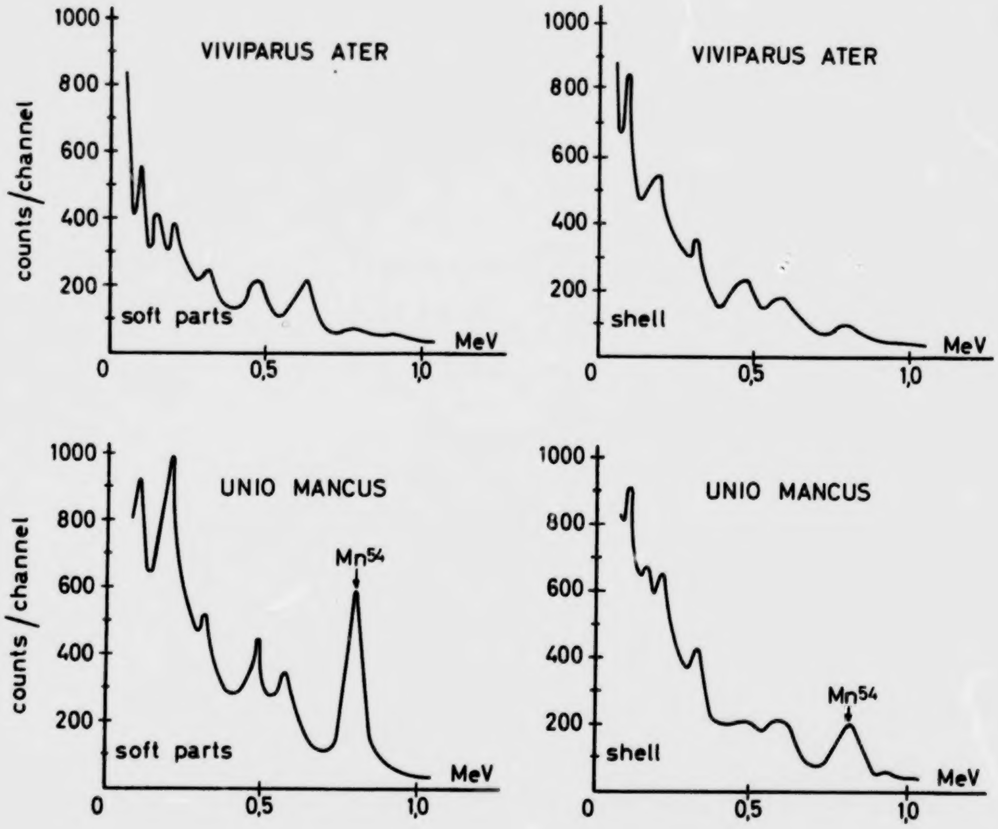

Figure 4. Gamma spectra of the shell and soft parts of Viviparus after and Unio Mancus.

weight. The total beta activity of benthonic organisms, as noted before, did not vary greatly at different depths. These two facts lead to the following supposition: 1) at different depths there are similar concentrations for the radionuclides utilized by the benthonic organisms and very different concentrations at different depth for those radionuclides discarded by the benthos; 2) the benthonic organisms cannot take up radioactive material after a certain level of radioactivity is reached.

The natural radioactivity has been increased in recent years by addition of fallout. It seemed interesting to ascertain if such an increase was detectable in the depth of the sediment. A core was collected from the sediment, therefore, at a depth of 313 meters (January, 1961) and subdivided into successive layers of three millimeters of thickness. The curve, drawn from the data obtained, shows clearly a peak of about 15 millimeters, perhaps corresponding to the high values calculated for the fallout of 1959 (Figure 6). The natural radioactivity values were those below a depth of 24 millimeters and were confirmed by other similar values obtained at greater depth inside the same core. The spectrum in Figure 7 shows the gamma emitters present in the surface layer of the sediment collected at a depth of 5 meters.

Table 4. CONCENTRATION OF MANGANESE-54 AND STRONTIUM-90 IN *UNIO MANCUS* AND *VIVIPARUS ATER* COLLECTED IN FOUR STATIONS OF LAKE MAGGIORE

Station	Mn^{54} (pc/g wet wt.)		Sr^{90} (pc/g wet wt.)
	Soft tissues		Shell *Unio* and
	Unio	*Viviparus*	*Viviparus*
Ispra	0.11	0	1.6
Fondotoce	0.25	0	1.6
Arona	0.30	0	1.6
Angera	0.40	0	1.6

Data on other lakes of Northern Italy (Varese, Commabbio, Alserio, Pusiano, Annone and Garda) led to very similar conclusions even though it became evident that each basin has its own characteristics for radioactivity as well as for any other ecological factor. For instance, the total beta activity of the shallow sediments varied from a maximum of 7.6 picocuries per gram wet weight for Lake Commabbio to a minimum of 2.7 picocuries per gram wet weight for Lake Maggiore; on the other hand, all values for the shallow sediments were lower in relation to those of deeper sediments in the same lake.

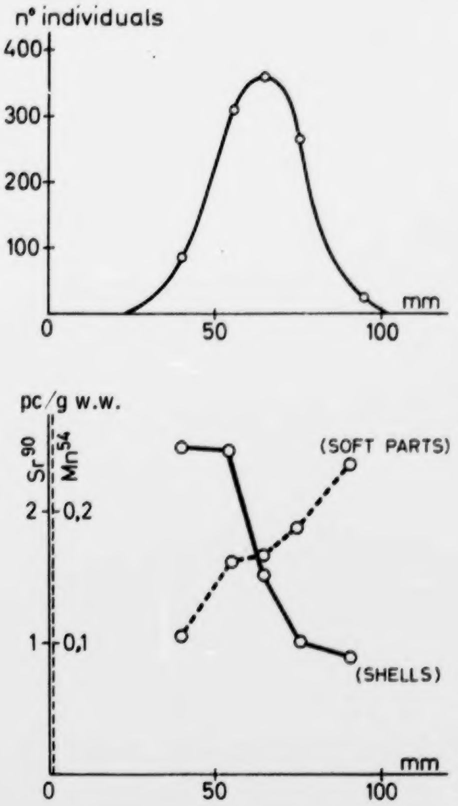

Figure 5. Distribution curve of the individuals of Unio collected in the different classes of length (age). Influence of the age on the concentration of Sr^{90} in the shell and of Mn^{54} in the soft tissues.

ACKNOWLEDGMENTS

Thanks are due to Dr. Margaret Merlini (Biology, EURATOM, Ispra) for her revision of the English test.

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The author expresses sincere thanks to the Health Physics Group, EURATOM, Ispra for its assistance in the measurements of radioactivity and for useful discussion.

Table 5. TOTAL BETA ACTIVITY IN DIFFERENT PARTS OF *UNIO* AND *VIVIPARUS*, AND MANGANESE-54 ACTIVITY FOR *UNIO*

Sample	*Unio mancus*		*Viviparus ater*
	Total beta activity	Mn^{54}	Total beta activity
Shell	1.99	0.00	6.74
Visceral sac	2.01	0.00	1.91
Mantle	2.69	0.19	
Gills	3.77	0.50	
Hepatopancreas			1.73
Operculum			2.10

Water depth in meters	pc/g w.w.
1	3
20	22
50	31
100	31
360	31

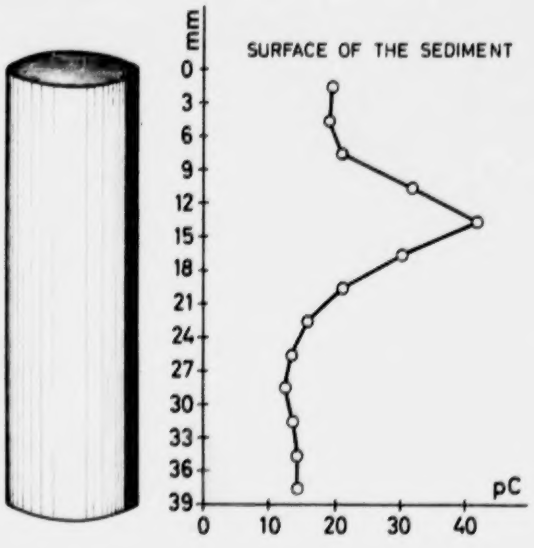

Figure 6. Total Beta-activity of the sediment of Lake Maggiore at different depths and in different layers of a core.

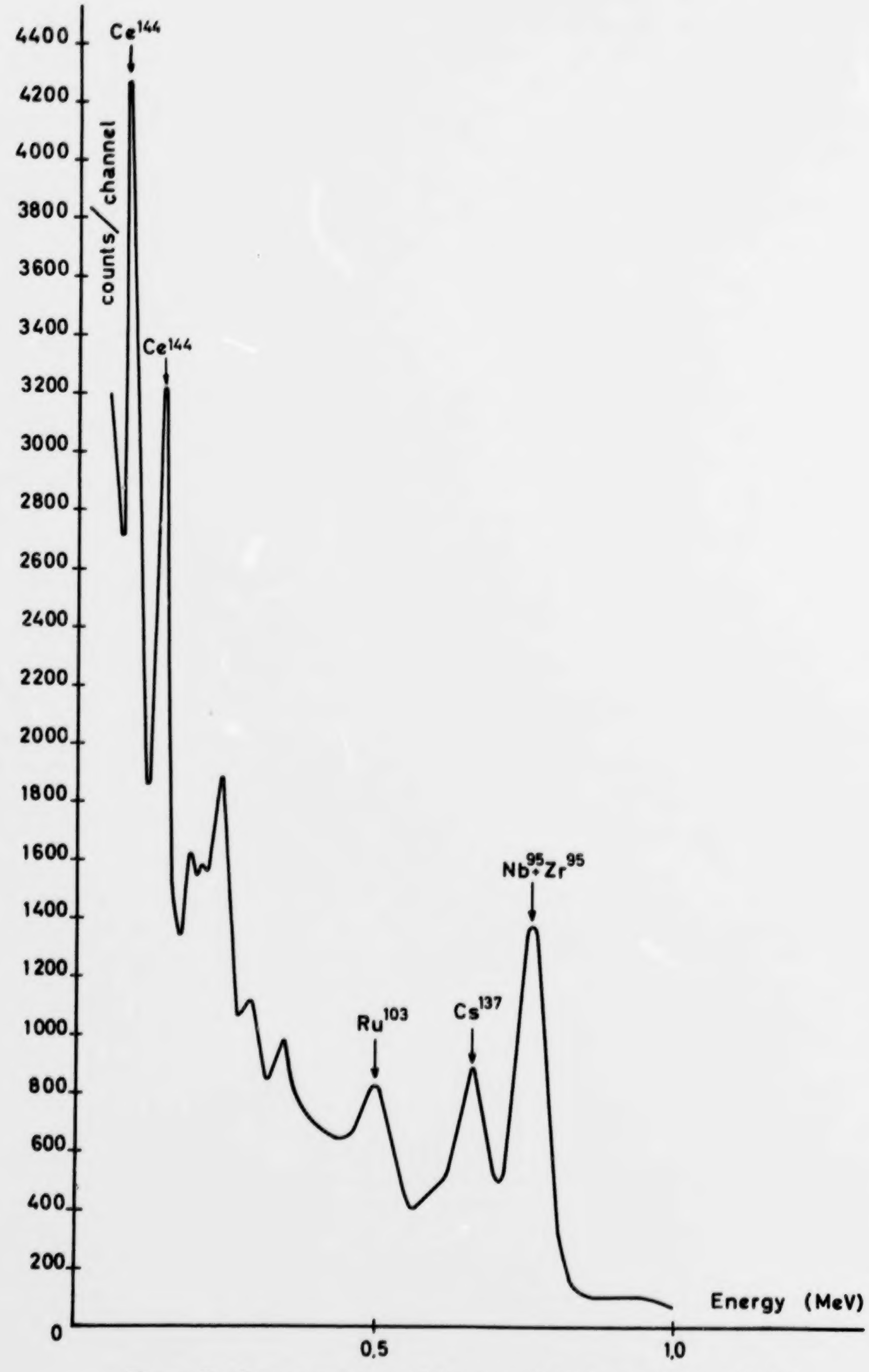

Figure 7. Gamma spectrum of the superficial layer of the sediment.

SUMMARY

Samples of plankton, larger aquatic plants, fishes, benthos, molluscs, and sediment collected in some lakes of Northern Italy (Maggiore, Garda, Alserio, Pusiano, Commabbio, Annone, and Varese)were examined to compare their radioactivity. Noteworthy was the presence of manganese-54, an activation gamma emitter, in the soft tissues of Lamellibranchs. The concentration of this radionuclide was very different in populations settled in different zones of Lake Maggiore. The highest level of radioactivity was found in the sediment; in this material total beta activity increased with increasing depth. The periphyton always showed higher values of radioactivity than the aquatic plants on which it lives. Season and zone had a very small influence on absorption of radioactivity by plankton, fish, and benthos; whereas age of the organisms played a very important role on this process both in molluscs and in fishes.

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ACCUMULATION OF RADIONUCLIDES AND THE EFFECTS OF RADIATION ON MOLLUSCS

Thomas J. Price *

Molluscan shellfish are among the most economically important organisms inhabiting estuarine waters. These animals feed by removing suspended particles from large volumes of water filtered through their gills. This filtered material includes phytoplankton, protozoa, bacteria, non-living detritus, and suspended inorganic particles. Most material is removed in particles entrapped in the mucous net of the gills; these mucoid particles are then transported along ciliary tracts to the labial palps where they are either ingested or rejected as pseudofeces. These mechanisms of filtering, transporting, and sorting are complex.

The principal food of molluscan shellfish is phytoplankton. These single-cell algae, because of their vast numbers and small size, present large surfaces for the direct adsorption of elements and their radionuclides (Goldberg, 1952; Rice, 1953; Rice and Willis, 1959). Since many molluscs are both filter feeders and bottom dwellers, phytoplankton with its associated elements and their isotopes is available as food both when alive in suspension and after death as settling estuarine detritus. It is known that phytoplankton will concentrate certain nuclides (Rice, 1953, 1956; Chipman, Rice, Price, 1958); thus shellfish may become exposed to an amount of radioactivity over that of the surrounding medium when feeding on radioactive plankton. Molluscan shellfish can obtain nutritive elements and their radioisotopes either from ingested food or from the surrounding water. Shellfish, by accumulating radioactivity, also may become an important link in the passing of radioactivity to higher trophic levels.

Estuaries in which a great many of the economically important molluscs are found have become potential areas of radioactive pollution. These bodies of water are collecting basins for drainage from the surrounding land masses. Pollutants reach estuaries either by leaching from the surrounding soil or in water from contaminated rivers. Within the past 15 years, the number of reactors and other possible sources of accidental release of radioisotopes into estuarine waters has increased. Many radioactive isotopes have become important because of their release in effluents from the various types of reactors. The increased number of licensed isotope users has created a problem of disposing of radioactive wastes. Already there are a number of nuclear ships adding to radioactive wastes released into marine waters. A large percentage of the increased radioactive fallout from nuclear detonations might possibly find its way into the oceans. Therefore, it is important that information be obtained so that the biological cycling of radioactive materials can be followed.

Radioactive isotopes have a common characteristic in that they emit ionizing rays and particles that are damaging to living plants and animals. Radiation occurs both from radioactive materials in the water and nuclides ingested by the aquatic organism. Selective accumulation of activity in certain tissues of organisms may result in localized damage. Harmful effects to the biota may be classified as somatic or genetic according to whether the effect damages the immediate physiological functions or tissues of the animal or involves the reproductive processes thus resulting in abnormalities in subsequent generations.

The accumulation of radioactive materials and the effects of radiation on marine organisms are of importance since they can affect man. There is a possibility that radioactive contaminants may reach man in high concentrations through seafood. Also, there is concern that the abundance of these marine organisms may be diminished from changes brought about by radiation injury.

The following is a report of experimental work carried out at the Radiobiological Laboratory of the Bureau of Commercial Fisheries, Beaufort, North Carolina, a cooperative project of the U. S. Fish and Wildlife Service and the Atomic Energy Commission.

The author wishes to express his appreciation to Dr. A. A. Armstrong, School of Textiles, North Carolina State College, Raleigh, North Carolina, for permission to use their cobalt-60 source.

The accumulation and retention of cesium-137, cerium-144, zinc-65, and gold-199 and the effects of radiation were followed on the hard clam, *Mercenaria mercenaria*, Linne; the oyster, *Crassostrea virginica*, Gmelin; and the bay scallop, *Aequipecten irradians*, Say. These animals were collected in the vicinity of the Radiobiological Laboratory and held in tanks of running sea water until used. All fouling organisms were cleaned from the shells prior to using the animals in experiments.

Cotton-filtered sea water having a salinity range from 29 to 35^{0}/oo and a temperature of 22 ±2^{o}C was used in all uptake experiments. The sea water for retention experiments was pumped directly from a source outside the laboratory; salinity and temperature varied with the tide and seasons.

Live animals were wrapped in polyethylene and measured for their contained radioactivity. Animal tissues were prepared for counting by rinsing in filtered sea water to remove loosely adsorbed surface activity. All radioactivity measurements were made with a well-type scintillation crystal attached to a conventional scaler. Size of the crystal used was determined by size of the animal. Radioactivity is reported as counts per minute per gram with appropriate corrections for geometry, background, and decay.

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Radionuclides may be grouped into two general classifications based on their origin. One includes fission products resulting from the splitting of the nucleus of the uranium atom or plutonium atom; the others are induced radionuclides formed by stable isotopes capturing neutrons. The radionuclides that occur from the fissioning of uranium are known, but induced radionuclides vary since they are isotopes of elements present in the area of the neutron flux. Some of these induced radionuclides have been found to be more important than the fission products as contaminants in radioactive wastes. Two of the fission products of concern in the oceans are cesium-137 and cerium-144. Zinc-65, which was found in marine fishes soon after the 1954 nuclear detonations in the Pacific, and gold-199 are two induced radionuclides that are discussed in this paper.

The rate of accumulation, levels of concentration, mode of entry, and assimilation of specific elements depend largely on their physical and chemical states in the medium and the physiological demands of organisms for the element. Elements and their radioisotopes are present in sea water either as particles or as ions. A radioisotope occuring in sea water in a particulate form may become associated with a molluscan shellfish by adsorption to its body surfaces or by ingestion into its digestive tract. Only a small quantity of the particulate nuclides may be retained as they pass through the digestive tract, since particles do not readily penetrate through semi-permeable membranes; however, the ionic radionuclides have little difficulty passing through tissue membranes and thus have the potential of being assimilated to higher levels.

CESIUM-137

Cesium is one of the alkali metals and is chemically similar to sodium, potassium, and rubidium (Hood and Comar, 1953). The radioisotope cesium-137 with a physical half-life of 30 ±3 years is found in sea water as a fission product chiefly in the ionic state. Cesium-137 is accumulated to relatively high levels by soft tissues of shellfish, thus making it an important radioactive contaminant since these soft parts are used as food by man.

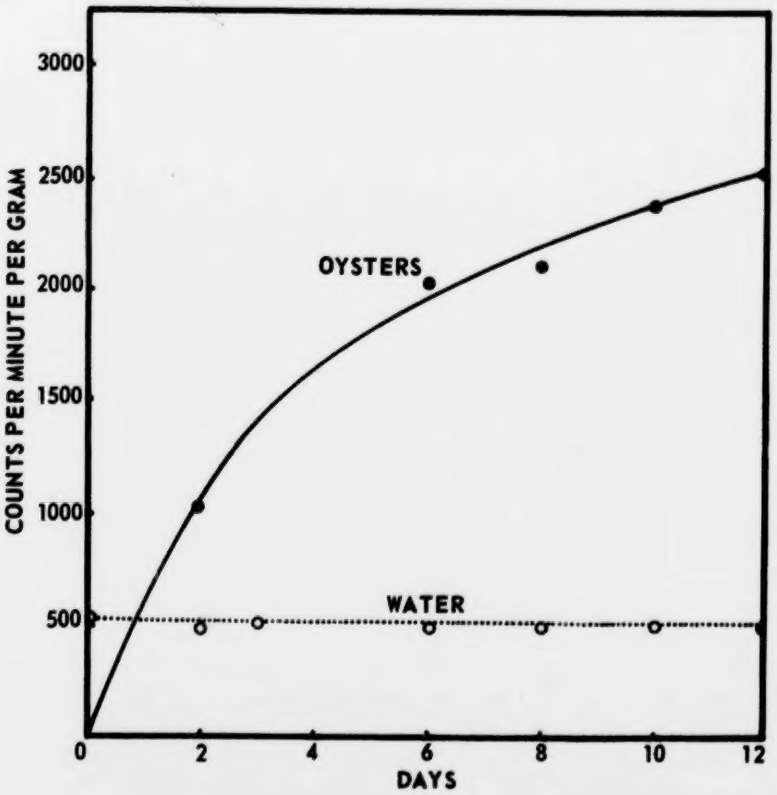

Figure 1. Uptake of Cs^{137} by oysters.

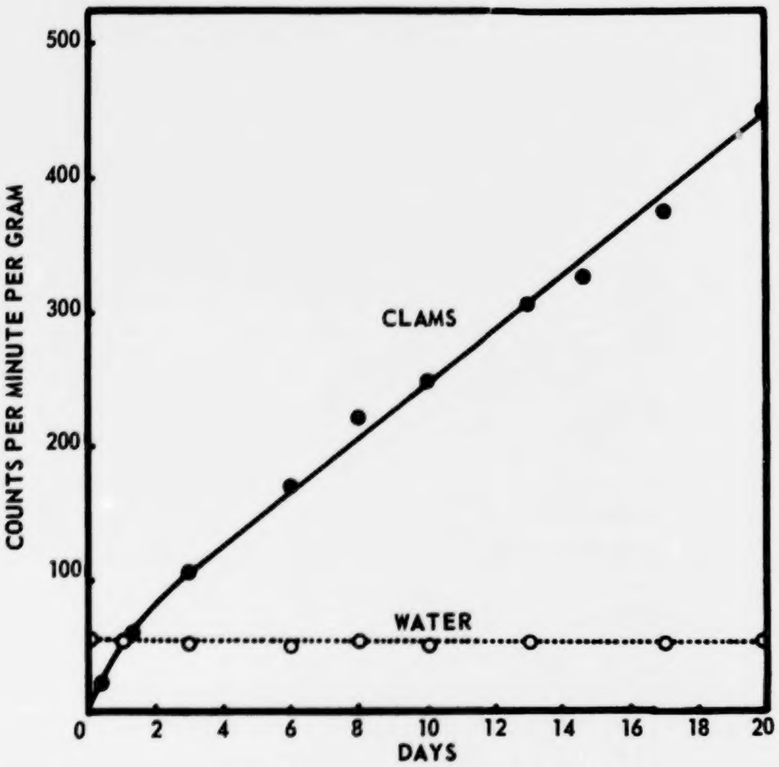

Figure 2. Uptake of Cs^{137} by hard clams.

Carrier-free cesium-137 was used as cesium chloride and was in secular equilibrium with its radioactive daughter product, barium-137. The time between sampling and measurement of activity was long enough for secular equilibrium to occur between the parent-daughter components, thus eliminating the effect of biological separation in the determination. The amount of cesium-137 that was accumulated or the concentration factor of this nuclide in the tissues of the animals was computed by dividing the activity per gram of animal or tissue by the activity per gram of sea water in which the animals were held.

The uptake of cesium-137 by oysters and clams is shown in Figures 1 and 2, respectively. Clams accumulated the radioisotope more than oysters. At the end of 20 days, the soft parts of the clams concentrated cesium-137 by a factor of six over the cesium-137 concentration in sea water. The clams were continuing the uptake of the isotope at the termination of the experiment, so it was not possible to determine the final concentration factor for clams. At the end of 12 days, the concentration factor of cesium-137 in the soft parts of the oysters was five; however, at that time the tissues of the oysters appeared to be entering into a steady state condition with the isotope.

It is necessary to determine the time required for biological elimination of the nuclides by molluscs, should they become polluted by radioactivity. This is essential to know so the shellfish will again be safe to use as food. The time required for a living tissue or organ or an individual to eliminate one-half of a given amount of substance through biological processes is referred to as the biological half-life. As an example, if a mollusc contains 1000 counts per

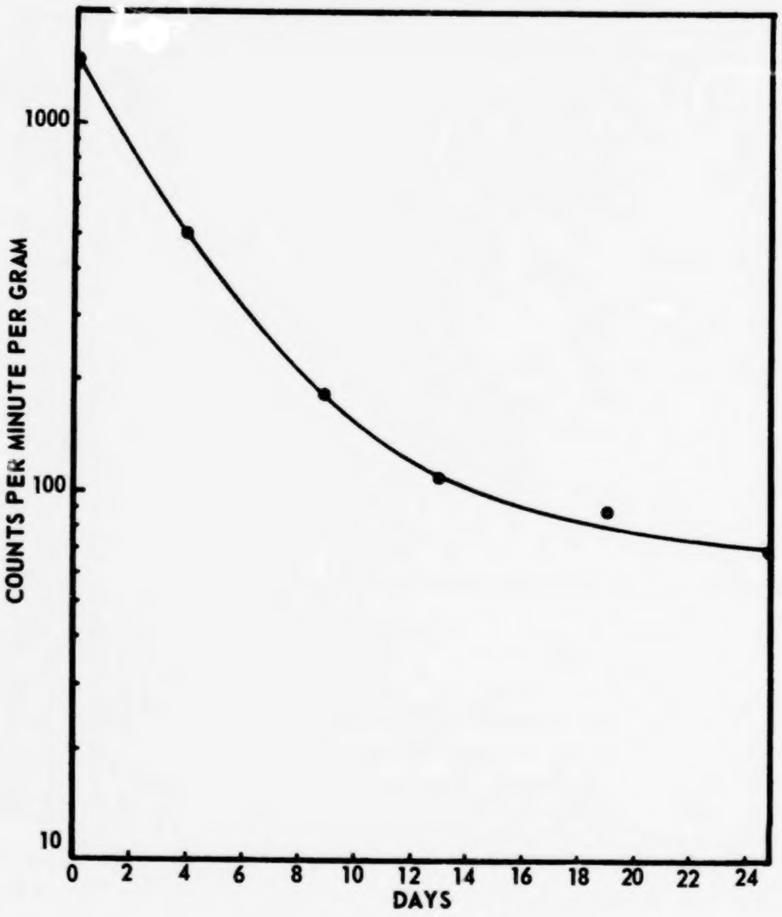

Figure 3. Loss of Cs137 by oysters.

minute initially and in 3 days the counts decrease to 500 counts per minute, this decrease of activity would give a biological half-life of 3 days for this particular animal and radionuclide.

Investigations were carried out to determine the loss of cesium-137 by oysters and clams (Figures 3 and 4). The soft tissues of oysters lost activity rapidly during the first 18 days, at which time the rate of loss decreased. After 25 days, only 4 percent of the total activity remained in the tissues. The loss of cesium-137 in clams was followed for 28 days. As

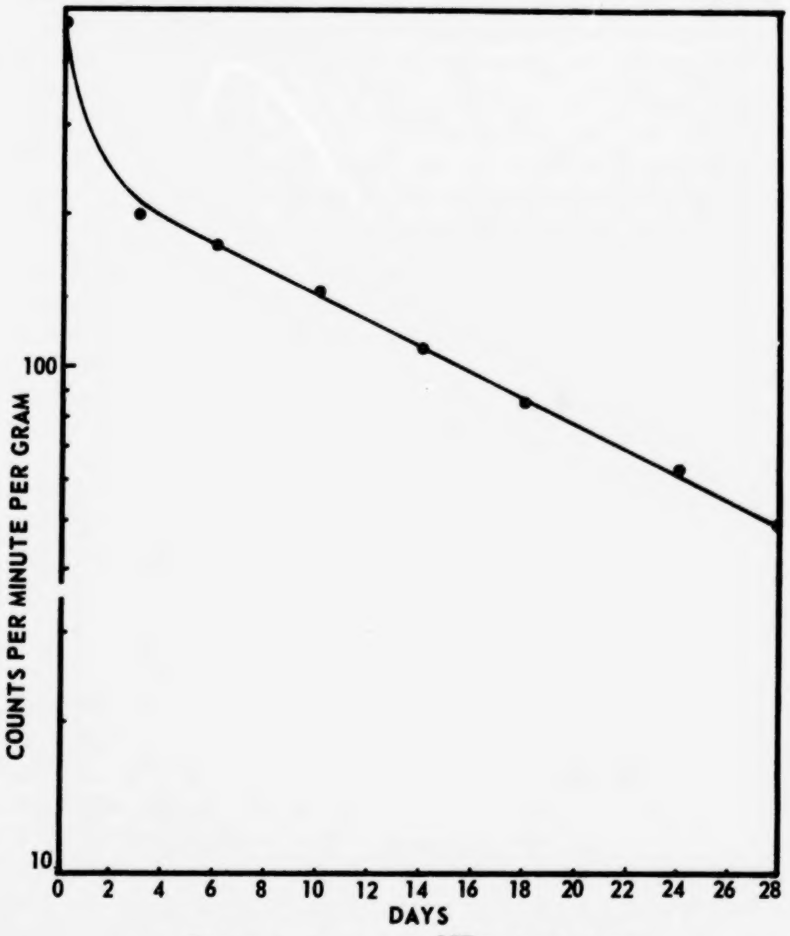

Figure 4. Loss of Cs137 by hard clams.

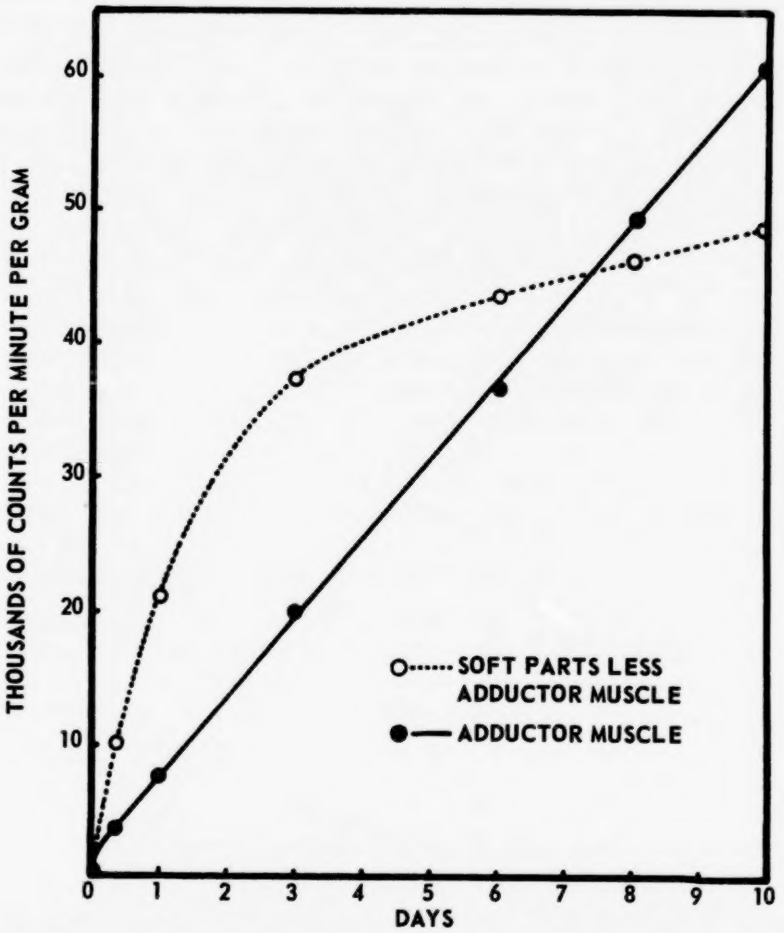

Figure 5. Uptake of Cs137 by bay scallops.

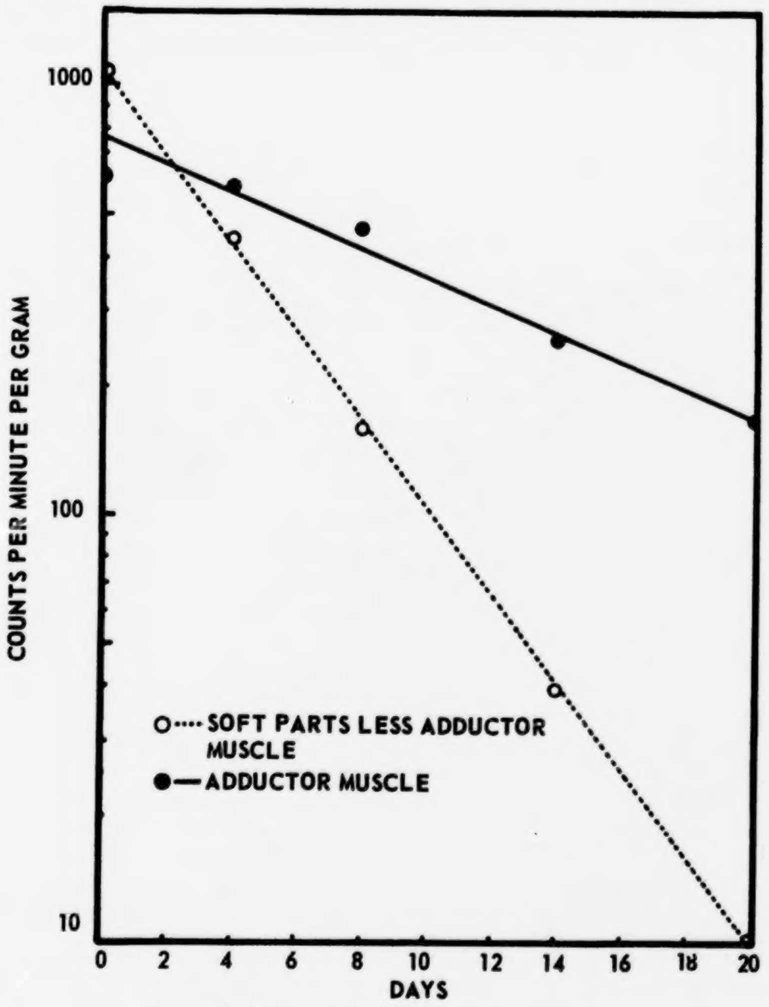

Figure 6. Loss of Cs137 by bay scallops.

with the oysters, a slower rate of loss occurred following the rapid initial loss. Twelve percent of the isotope remained in the clams after 28 days, which was higher than for the oysters after the same time interval. This residual activity remaining in the tissues of the clams and oysters was possibly the result of the nuclide becoming complexed in chemical compounds within the tissues.

Accumulation and loss of cesium-137 was interpreted as having been influenced by muscle and the remaining soft parts. Separate analyses of these two components were made of the uptake and loss of cesium-137 by the bay scallop. The adductor muscle of the scallop is the only portion used dietetically in this country. It is important to know the rates of uptake and loss of cesium-137 by this tissue. Radionuclides entered the tissues rapidly at first, with the final level being higher than that of clams (Figure 5). There was a more rapid uptake by the soft tissues other than muscle, but in time the highest concentrations were reached by the muscle. The concentration of cesium-137 in the muscle tissue after 10 days was 10 times that in sea water and steadily increased, while that in the visceral mass was concentrated 8 times and leveled off. It is apparent that muscle is the higher concentrator of the nuclide.

The visceral mass of the scallop lost cesium-137 faster than the adductor muscle (Figure 6). After 20 days, 25 percent of the activity remained in the muscle while only 8 percent remained in the visceral mass. The biological half-life of cesium-137 in the muscle was 13 days and in the visceral mass 3 days. It is noted that the line illustrating the loss of activity by the muscle is a regression line of anti-logarithms of the counts.

The different rates of uptake and loss of cesium-137 by the muscle tissue and visceral mass of the scallop indicate how these two components influence uptake and loss of this nuclide in an organism. This relationship is a rapid initial uptake or loss as influenced by the visceral mass, and then a longer continuous uptake or loss as influenced by the muscle tissue.

CERIUM-144

The physical state of a fission product in sea water controls to a great extent its mode of entry and subsequent assimilation by marine organisms. Greendale and Ballou (1954) determined the physical states of fission product elements in sea water by simulating an underwater bomb detonation. They found for cerium-144 that approximately 2 percent was ionic, 4 percent collodial, and 94 percent particulate. This large amount of cerium-144 in sea water in particles influences availability of this radioisotope to molluscan shellfish, as evidenced in the following experiments.

The concentration of cerium-144 by tissues of clams and oysters was determined when the nuclide was available to the animals as particles in the medium or associated with known populations of *Nitzschia closterium* cells. Cells from 1 liter of culture were fed daily to the animals. To maintain optimum volume of water to animal ratios as clams and oysters were removed for sampling, both the cell populations and the volume of medium containing the cells to be fed the animals were decreased proportionally. The uptake of the radionuclide was slow and continuous through the tenth day, after which it fluctuated in the tissues for the remaining 12 days of the experiment. The clams reached their maximum levels of accumulation after 20 days, with 23 percent of that made available daily being accumulated. It is assumed that the remainder of the cerium-144 available during the experiment adsorbed to the shells of the clams and oysters and to the container, as water samples taken before changing the medium each day did not contain any significant amounts of cerium-144.

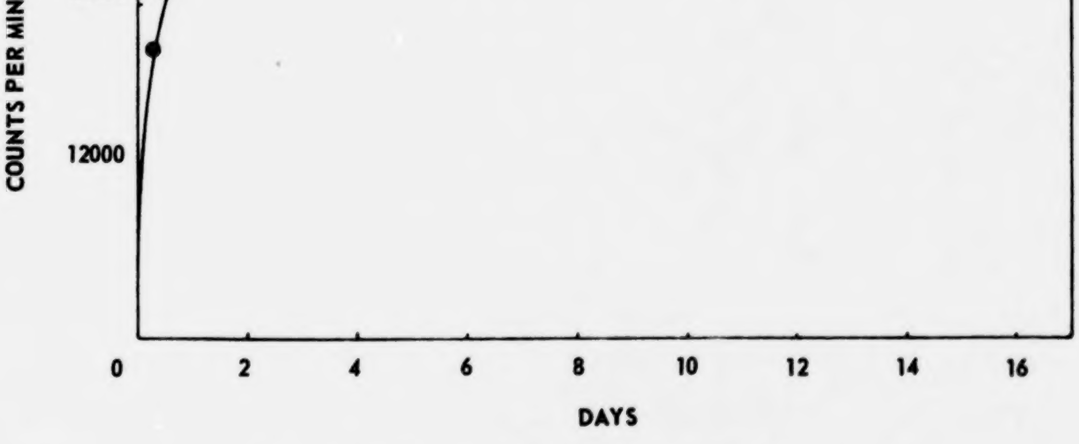

Figure 7. Uptake of cerium-144 from water by clams.

In experiments on the uptake of cerium-144 by clams, the animals were not sacrificed but were periodically removed from the radioactive water long enough for the activity in the entire animal to be measured. This method of repeatedly determining activity in the same animal throughout an experiment decreases effects of individual variations. Initially uptake of the radionuclide was rapid, but began to decrease on the third day (Figure 7). This diminished rate of uptake was undoubtedly caused by a decrease in the availability of cerium-144 in the water. Only 11 percent of the original activity remained in the water after 3 days and upon termination of the experiment after 12 days only 4 percent remained in solution. This removal of the nuclide from solution affected the availability of the isotope to the clams, and thus possibly influenced the levels to which cerium-144 was accumulated.

Investigations were carried out to determine cerium-144 uptake and distribution by shell, liquor, and meats of the clams. Animals were removed periodically, dissected, and the activity in the three components was determined. Shells contained the highest level of activity, followed by meats, and liquor (Figure 8). Radioactivity of the shell was largely surface adsorption of cerium-144, while activity associated with the meats was possibly the effects of particles adhering to tissue surfaces and present in organs and structures connected with the digestive tract. Only a small percentage may be assimilated since particle size would be a limiting factor in their absorption through the walls of the intestinal tract.

It is essential to know the time required for biological elimination of radioactive pollutants by molluscs to conclude when they again will be available as food for man. To understand the metabolic role of cerium-144 more fully, the following experiments were conducted with special emphasis on long term retention and whole animal counting. To determine the rate of loss by clams, they were immersed in cotton-filtered sea water containing cerium-144 and after a suitable time were transferred to tanks of rapidly flowing sea water. The initial loss of cerium-144 was rapid during the first 45 days, after which the loss was reduced (Figure 9). Cerium-144 remaining after 195 days represented 20 percent of the original activity in the animals. It is possible that activity remaining at this time is either complexed by the metabolic processes of the animals or securely adsorbed to body surfaces.

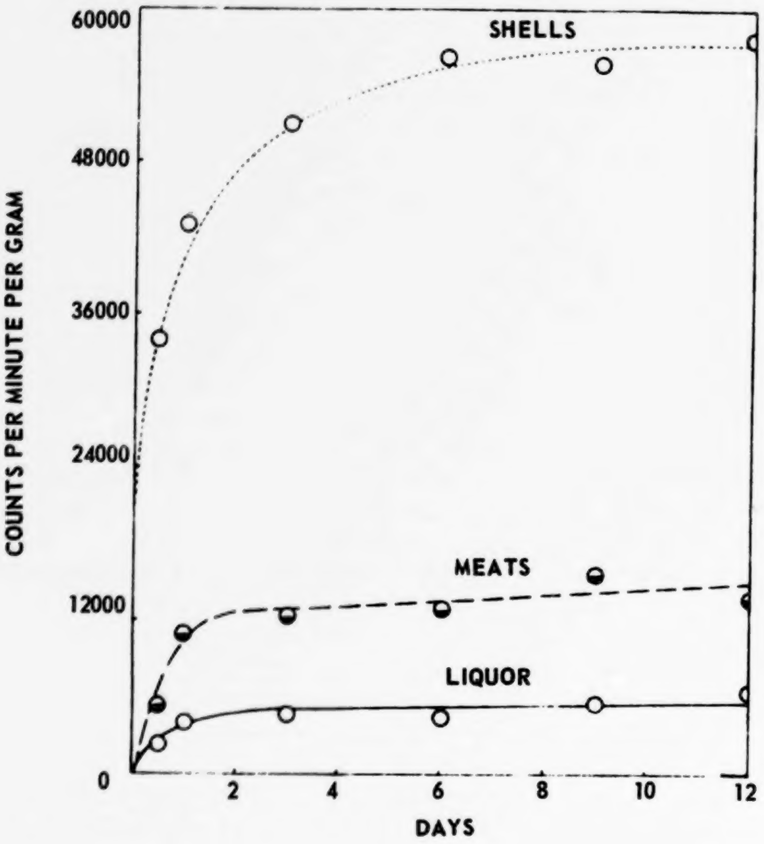

Figure 8. Uptake of cerium-144 by component parts of clams.

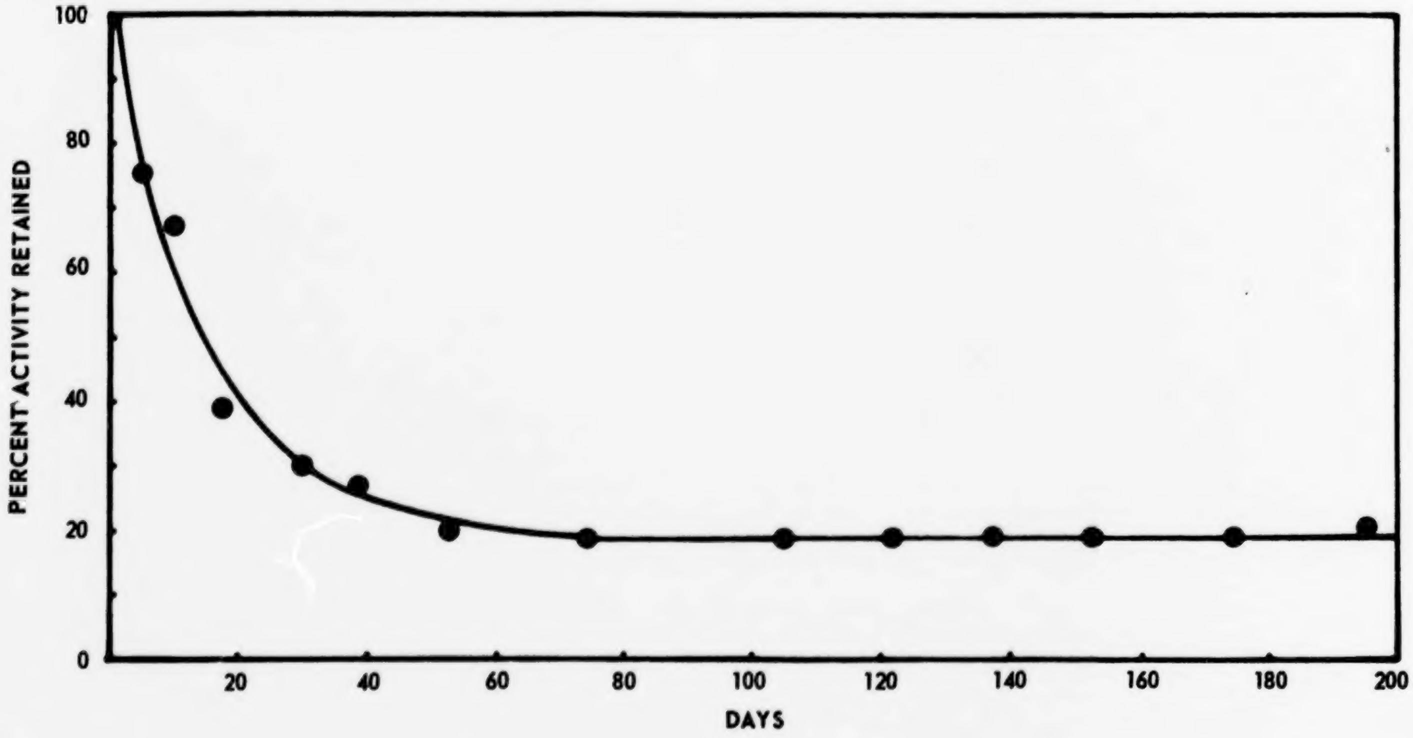

Figure 9. Loss of Ce^{144} by the hard clam.

The rate of loss of cerium-144 by the bay scallop was observed for 35 days. The animals were removed from sea water and separated into shell, visceral mass, and adductor muscle. The loss of the radionuclide in all three tissues was rapid in the beginning, followed by a reduction in the rate of loss (Figure 10). After 35 days, the shells had lost 55 percent of the original activity; muscle, 62 percent; and the visceral mass, 89 percent. It appeared that any further loss of activity from these three components would be small.

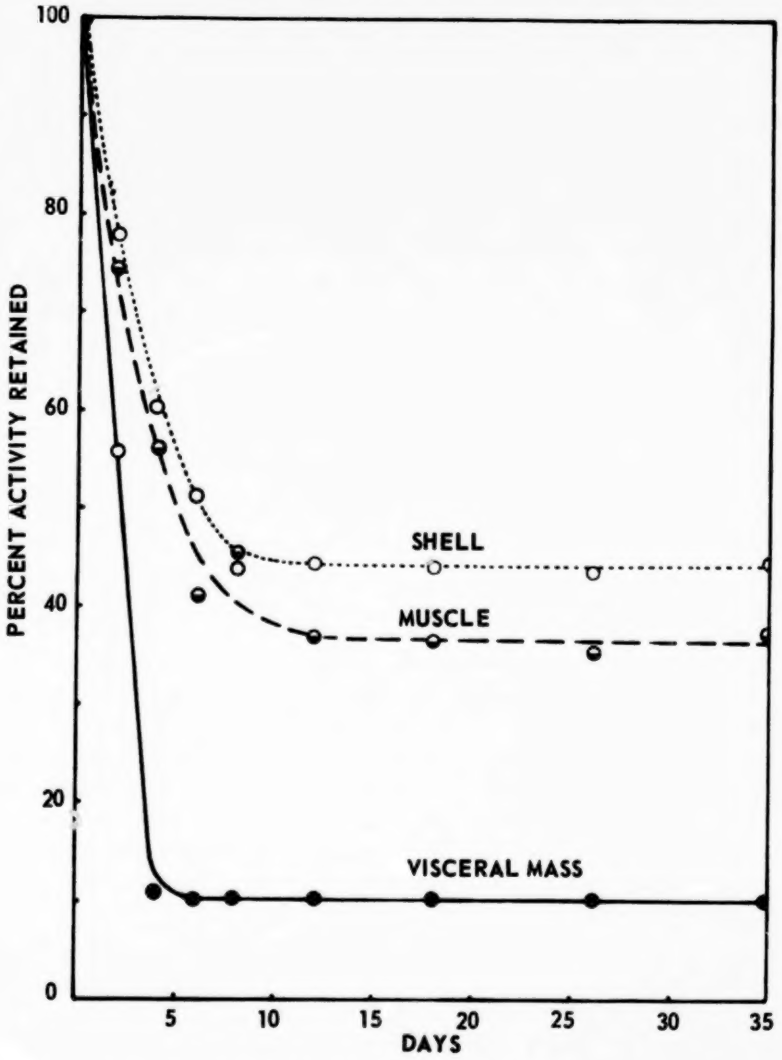

Figure 10. Loss of Ce144 by components of the bay scallop.

ZINC-65

In the past most data on uptake, accumulation, and loss of radionuclides by marine organisms has concerned single species held in confined volumes of water. This is not a very satisfactory manner of determining the relationship that would exist between the radioactivity of the water and that of the organism in nature, since the volume of water per organism would be a controlling factor of the experiment. If five animals are placed in ten liters of water and remove a large amount of the activity, they will have a higher concentration factor than would have occurred if ten animals had been placed in the same volume of water. Thus, sufficient volumes of water should be used to minimize the effects of animals drastically reducing activity in it. Results of our tank experiments reveal not only the effects of contamination on an entire community, but also permit evaluation of an individual in relation to the ecosystem. By increasing the volume of water in an experiment, it is possible to conduct experiments of long duration due to the improved physiological condition of the organisms; thus both chronic and acute contamination can be investigated.

The universal occurrence of zinc in all living matter and its role as an essential nutrient for plants and animals is firmly established (Vallee, 1959). The presence of zinc-65 in marine organisms from the Pacific Ocean following nuclear test detonations has been reported by Seymour *et al.*, 1957; Lowman, Palumbo, and South, 1957; and Welander, 1957. This neutron-induced radionuclide is produced by nuclear changes of the stable element and occurs in marine waters principally in the ionic state. Zinc-65 can also be introduced into the oceans as part of corrosion products present in the coolant water of nuclear reactors.

Molluscs in general have a higher zinc content than other invertebrate groups (Vinogradov, 1953). Thus, these shellfish are important as transfer organisms of zinc-65 through food webs to man. The uptake and accumulation of zinc-65 by oysters and clams was followed in an experimental environment consisting of 1200 liters of cotton-filtered sea water continuously circulated in a fiberglass tank. Enough carrier-free zinc-65 was added to the water to give a concentration of 8×10^{-5} microcuries per milliliter. The animals were removed periodically for counting and then returned to the radioactive medium. A conversion factor was needed to compensate for differences in geometry

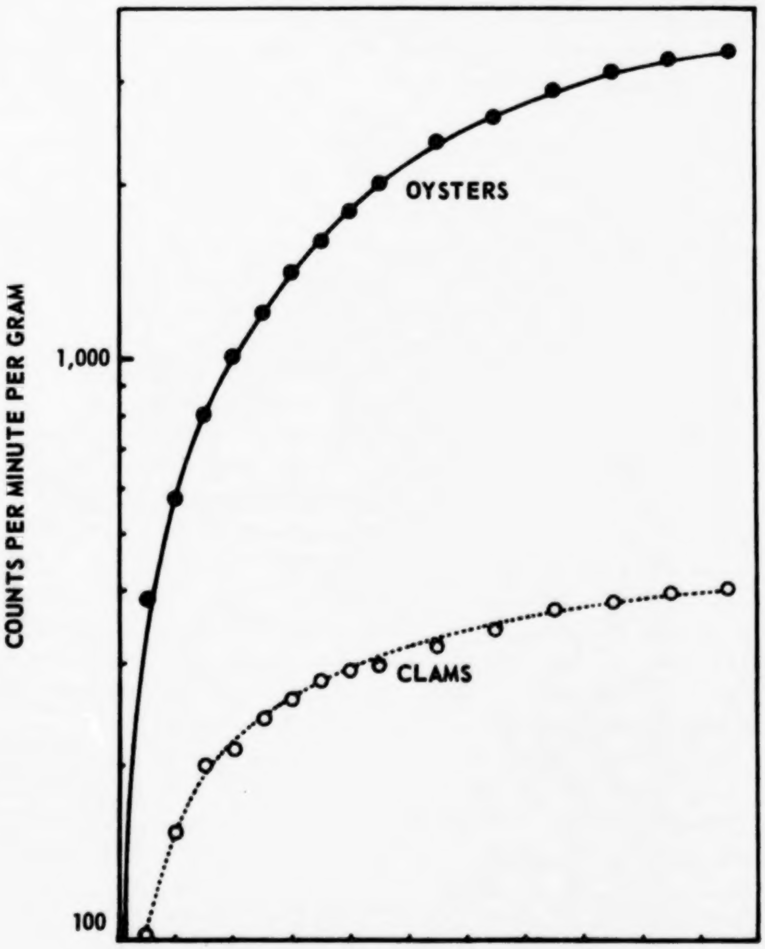

Figure 11. Accumulation of Zn^{65} by oysters and clams in an experimental marine environment.

encountered in comparing activity in the animals and in the water. This factor was derived by first measuring the activity in the animals directly in a whole animal counter, secondly, digesting the animals in acid, and finally diluting to the same volume as that of the sea water that was measured for activity. An average of three measurements yielded a factor of 0.77; that is, the sample counted 23 percent less when dispersed in water, which was possibly the result of the shielding effect of the water. Clams accumulated zinc-65 at a slower rate than oysters on a unit weight basis (Figure 11). At the completion of the experiment, however, it was observed that these two curves if extrapolated would continue upward, indicating a gradual but reduced uptake of zinc-65 in both clams and oysters. Thus, oysters concentrate zinc-65 to higher levels than clams. This increased amount of zinc-65 in oysters was possibly caused by a greater physiological demand for this element than clams, as is indicated by the natural zinc content of these two animals.

GOLD-199

Molluscan shellfish are particularly susceptible to contamination by tagged radioactive sediments, since these animals inhabit the bottom of estuaries and feed by filtering particles from large volumes of water. Radioisotopes such as gold-199 have been utilized as tracers in estuarine waters to determine the movements of these sediments (Krome, 1960). Gold-199 was the isotope of choice because of its short half-life, adsorptive power, and detectability.

To investigate uptake and accumulation of gold-199, clams and their separated shells were placed in radioactive water. Another group of clams was placed in bowls containing montmorillonite clay and these were immersed in the sea water. The clams and separated shells in the water were removed periodically to determine their radioactive content, while those in clay were counted at the end of the experiment so as not to disturb the substrata.

The uptake of activity by both clams and shells was rapid during the first 14 days, followed by a much slower rate of uptake throughout the remaining time of the experiment (Figure 12). Clams contained 3.6 times as much activity than shells upon termination of the experiment. This difference was shown to be significant through a comparison of means by a t-test. The higher concentration in the clams was apparently the result of adsorption of the nuclide to their body surfaces and possibly some contribution from metabolic processes. The clams in the montmorillonite clay contained 38 percent less activity after 23 days than those in the water. This lower activity conceivably was caused by the clams burrowing in the clay, preventing them from obtaining as much activity as those in the active water.

RADIATION EFFECTS

The importance of the effects of ionizing radiations on marine organisms has increased since the oceans have become a convenient area in which to dispose of radioactive waste materials and to test nuclear weapons. This potentially harmful radiation may originate either from the fission process itself or from ingested by-products of the fission process. Thus, a reduction of harvestable living resources of the sea could conceivably occur from the effects of atomic radiations on organisms that are utilized in commercial fisheries or by a reduction in those fish that are utilized as food by these economic species.

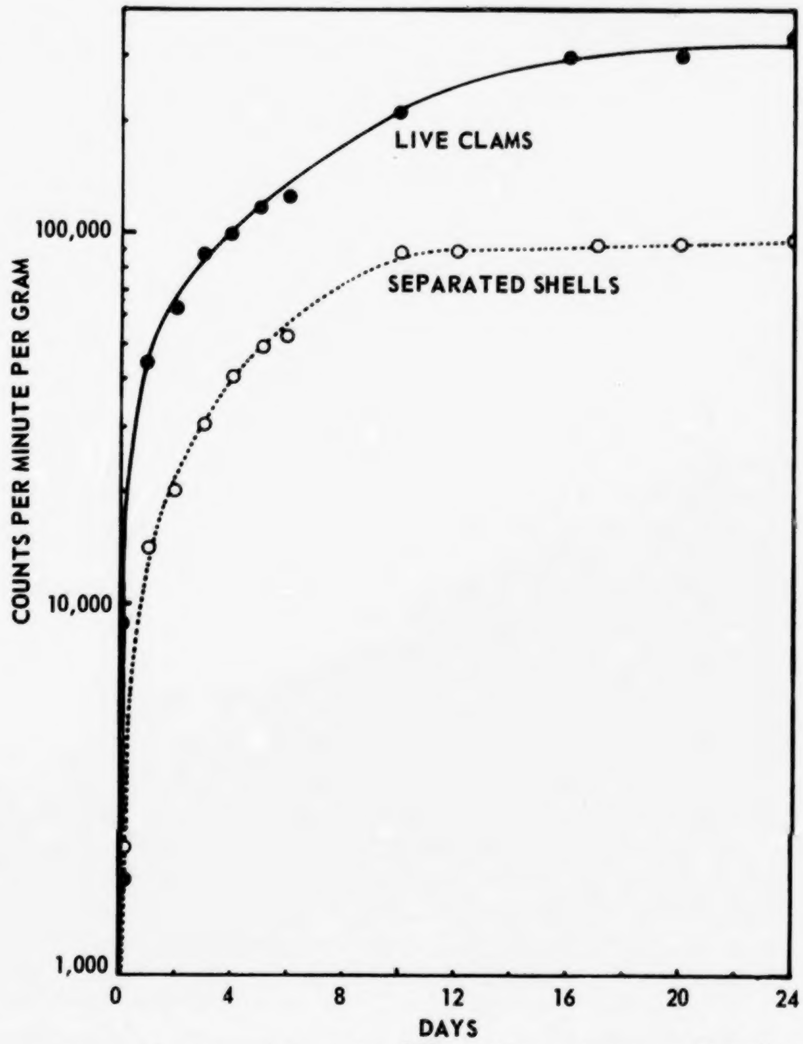

Figure 12. Accumulation of Au^{199} by clams and separated shells.

The following experiments were undertaken to determine the lethal doses at which 50 percent (LD$_{50}$) of the oysters and clams died.

All animals were irradiated with a cobalt-60 source with a dose rate of 350,000 roentgens (r) per hour. The radiation chamber was a cylindrical cavity 6 inches in diameter by 8-1/2 inches high. Polyethylene containers were used to hold the animals while being irradiated. After irradiation the animals were returned to tanks of rapidly flowing sea water where daily checks were made for mortalities.

Fifty animals composed each group receiving the different dosages of radiation. Oysters were exposed to 5,833; 11,666; 23,332; 46,664; 93,328 and 186,656 roentgens. Irradiation of clams was in similar groups except for three groups irradiated at 116,600, 139,992; and 163,324 roentgens.

The cumulative mortality of the various groups of clams is shown in figure 13. There were no mortalities in the control groups of clams or oysters. The LD$_{50}$ for clams in the various dose groups are: 186,656 r - 5.5 days; 163,324 r - 4.5 days; 139,992

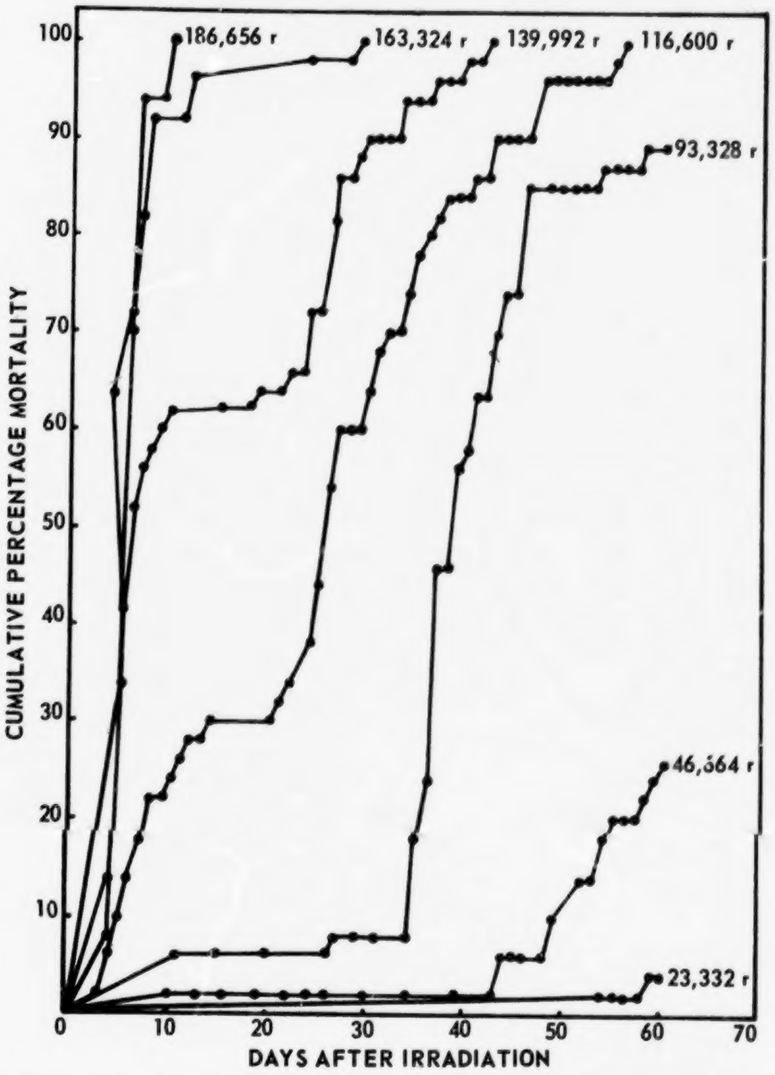

Figure 13. Daily cumulative percentage mortality of Mercenaria mercenaria subjected to graded doses of cobalt-60 gamma radiation. (No mortalities in control).

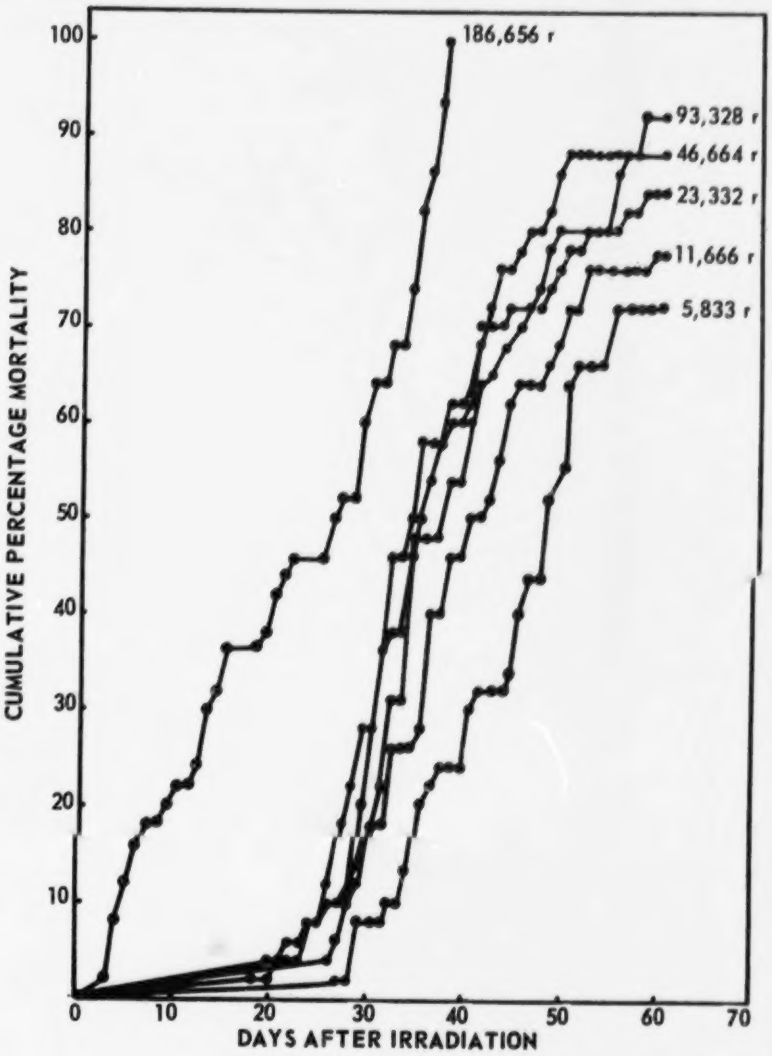

Figure 14. Daily cumulative percentage mortality of Crassostrea virginica subjected to graded doses of cobalt-60 gamma radiation. (No mortalities in control).

r - 6.5 days; 116,600 r - 25.5 days; 93,328 r - 38.5 days. The remaining groups of irradiated clams had not attained a 50 percent mortality after 60 days.

The LD_{50} for oysters (Figure 14) in the various radiation groups is: 186,656 r - 26 days; 93,328 r - 34 days; 46,664 r - 36.5 days; 23,332 r - 35 days; 11,666 r - 40 days; and 5,833 r - 48 days.

The physical state of materials introduced into the sea controls the role they play in the metabolism of marine animals. Radioactive contaminants occur either as particles or in solution. Particles may go into solution depending on the physical and chemical properties of a particular body of water. The dissolved substances may be precipitated as particles of colloidal or larger size either by co-precipitation with other elements, by sorption on organic or inorganic particles already in the sea, or by interaction with other sea-water constituents. Thus, both dissolved materials and particles may be ingested by organisms and enter into the biochemical cycle.

Availability of radionuclides for uptake by shellfish is dependent in part on the chemical nature and physical state of the nuclides when present in sea water. Radionuclides can further be categorized according to the methods by which they are produced, that is whether they are products of the fission processes or neutron-induced. The four radioisotopes used in these investigations thus have the following classifications: Cesium-137, ionic; cerium-144, particulate (both fission products); zinc-65, ionic; gold-199, particulate (both induced radionuclides). Given data indicate that the uptake of all the nuclides by these molluscs was basically the same, an initial rapid acceptance of the element followed by a continued but reduced rate of uptake. This initial rapid uptake appears to be a combination of physical adsorption on body surfaces and metabolic processes of the animals. The later, continued reduced rate of uptake is an effect of the animals' metabolic action, since saturation has now been achieved through sorption. Loss of these four nuclides showed a similar pattern as that of the uptake.

Organisms take up from their environment and food and incorporate into their bodies the elements needed for their maintenance, growth, and reproduction. The proportions of the essential elements required by organisms are different from the proportions in the environment. This results in concentrations of some elements in the biosphere. Molluscs differ in their uptake of the four isotopes mentioned. The concentration factor of an element has already been defined as the amount of an element in an organism in ratio to the quantity in an equal weight of water. Concentration factors vary with season, with the concentration of an element in water, with the concentration of other metabolically similar elements, with the age of an organism, and with a number of other such factors. Also, the concentration of a nuclide depends both upon the metabolic need of the particular organism for the element, and the tissue preference of the nuclide. Cesium-137 is accumulated to relatively high levels by the soft tissues other than muscle, but in time the highest concentrations are reached by the muscle. It was found that clams concentrated cesium-137 to a higher level than oysters, which is in agreement with the differences in their body structure as regards muscle tissue.

Whether or not the radionuclide occurred in the ionic or particulate state in sea water made little difference in its uptake by the three groups of shellfish. If the particulate radionuclides cannot be metabolized by the animals they may adsorb to body surfaces or associate with the organs and structures connected with the digestive tract. Such an accumulation of radioactive particles may be important for purposes of passage through food webs to man. The fact that the adductor muscle of scallops and all the soft tissues of oysters and clams are eaten allows for the ingestion of many radionuclides that may not be metabolized and incorporated into the tissues of these shellfish. Many nuclides are concentrated by the shells of these animals. Accumulation of radioactivity by shells may be important since they are placed on the bottom by oystermen as cultch to which the young oyster spat attaches; they are also used in many other industries.

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ACCUMULATION OF RADIONUCLIDES BY AQUATIC INSECTS

J. J. Davis *

The literature contains results of numerous studies of insect control, physiology, and ecology in which radionuclides were used as tags. The relatively few such studies that have dealt with aquatic insects pertained mainly to the use of radioactive "tags" in studying dispersion of economically important forms such as mosquitoes and black flies (Hassett and Jenkins, 1949; Bugher and Taylor, 1949; Jenkins, 1949; Thurman and Husbands, 1950; Shemanchuk, Spinks, and Fredeen, 1953; Fredeen, et al., 1953; Gillis, 1962).

The section of the Columbia River that is within the Hanford Reservation in southeastern Washington provides a unique opportunity for studying accumulation and cycling of radionuclides in natural aquatic communities. More than 60 radionuclides have been identified in the aqueous effluents that are continuously discharged into the Columbia River from some of the Hanford reactors. Many of these, because of their infinitesimal initial amounts in the effluent waters and extremely short radioactive half-lives, are not detectable in the river. About 20 of them have been measured in some river organisms collected within 1 mile downstream from the reactor outfalls. (Davis, et al., 1958).

Insects living in the river below reactor outfalls are many times more radioactive than is the water they inhabit. For example, larvae of the caddis fly *Hydropsyche cockerelli* Banks collected within 3 miles downstream from reactors, commonly have a gross beta radioactivity 1,400 times greater than that of the water. Due to metabolic selection, the quantities of radionuclides accumulated by insects are different from those of the water. The five most abundant radionuclides in insect larvae, in decreasing order, are phosphorus-32, copper-64, chromium-51, zinc-65, and sodium-24 as compared to copper-64, neptunium-239, sodium-24, manganese-56, and chromium-51 in millipore-membrane-filtered river water from the same collection site. A typical accumulation factor pattern of these radionuclides in *Hydropsyche cockerelli* larvae during the late summer (water temperature about 18°C) is shown in Table 1.

An organism's location in the food web, its trophic level, may affect the amount of radionuclides accumulated. The magnitude of this influence is dependent upon several factors. Of paramount importance is the time required for the radioactive material to pass from water to the organism in question. The accumulation efficiency and transport time of radionuclides are, in turn, dependent upon metabolic demand and the biological turnover rate (biological half-life) of the element for each component in the food web (Davis and Foster, 1958). And last, the degree to which transport time influences the efficiency of transfer depends upon the physical half-life of the radioisotope involved. Long-lived radionuclides such as strontium-90 (28-year half-life), cesium-137 (30-year half-life) or carbon-14 (5770-year half-life), of course, would be essentially unaffected.

An example of the pronounced differences that commonly occur in amounts of radionuclides accumulated by different species of insects from the same habitat is shown in Table 2. These differences are caused by an interplay of ecological and metabolic factors. The most influential factors are probably food habits and biological turnover rates (biological

Table 1. RADIONUCLIDE ACCUMULATION FACTORS FOR CADDIS FLY LARVAE FROM COLUMBIA RIVER

Radionuclide	Physical half-life	Accumulation factor (radioactivity/g larvae, live weight) radioactivity/ml water
P^{32}	14.3 days	100000
Zn^{65}	245 days	20000
Cr^{51}	27.8 days	3000
Cu^{64}	12.9 hr	400
Na^{24}	15 hr	80
Np^{239}	2.3 days	30
Mn^{56}	2.58 hr	not detectable in insect

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* Biology Laboratory, Hanford Laboratories, General Electric Company, Richland, Washington.

(Work performed under Contract Number at (45-1)-1350 between the General Electric Company and the Atomic Energy Commission, Division of Biology and Medicine.)

Table 2. RADIONUCLIDES IN PLANKTON AND IMMATURE INSECTS FROM THE COLUMBIA RIVER NEAR HANFORD, FEBRUARY, 1957
(In units of pc/g wet wt.)

Radionuclides	Physical half-life	Plankton	Chironomid larvae (Hydrobaeninae)	Chironomid larvae (Tendipedinae)	Caddis fly larvae (*Hydropsyche*)	Caddis fly larvae (*Glossosoma*)	Caddis fly larvae (*Leptocella*)	Black fly larvae (Simuliidae)	Aquatic moth larvae (*Argyractis*)
Na^{24}	15 hr	1800	390	98	120	140		550	
P^{32}	14.3 days	11000	17000	2000	4200	4000	780	6800	3200
Sc^{46}	84 days	3100	21	33	56	58	39	36	77
Cr^{51}	27.8 days	73000	1100	1100	860	1000		1600	1200
Mn^{54}	314 days	31		26	100	20			62
Fe^{59}	45 days	2800		20	19	26			
Co^{60}	5.27 yr	37	9	5	30	21			46
Cu^{64}	12.9 hr	30000	1900	120	310	1200	390	3900	840
Zn^{65}	245 days	7300	980	820	730	1600	1100	570	3500
As^{76}	26.5 hr	14000			150			950	
La^{140}	40.2 hr	11000		43	170			430	80

half-lives). The importance of the food web position of the organism may be demonstrated by comparing the radionuclide content of plankton, *Hydropsyche*, and *Leptocella*. *Hydropsyche* feed mainly upon phytoplankton, whereas the *Leptocella* are dominantly detritus feeders, which places them at a higher position in the food web. Black fly larvae, which are plankton feeders, and Hydrobaeninae larvae, which reside in and consume periphyton, occupy the second trophic level. The Tendipedinae larvae, including *Chironomus decorus* Johannsen and *C. flumidus* Johannsen, occupy a different ecological niche in the same general habitat. These forms burrow in fine sediments and form tubes on the undersides of stones implanted in the sediments. They apparently feed upon miscellaneous organic debris that happen to be deposited at the orifices of their tubes.

When insects metamorphose from immature stages to adults, there is generally a reduction in their content of certain radionuclides. This is demonstrated by the adult to larvae radionuclide ratio for *Hydropsyche* in Table 3.

The radionuclide concentrations in insect larvae of the Columbia River follow definite seasonal cycles. Highest concentrations generally occur during the summer; Table 4 compares the radionuclide content of *Hydropsyche* larvae during summer and winter. The lack of measurements for zirconium-95-niobium-95, ruthenium-103, barium-140, and neptunium-239 in the larvae collected during the winter does not indicate the absence of these radioisotopes at that time, but is due to lack of an adequate sample to permit their detection. The degree of difference between summer and winter concentrations is generally most pronounced for radioisotopes of elements that are readily metabolized, such as sodium-24, phosphorus-32, and copper-64.

Table 3. RADIONUCLIDE RATIOS BETWEEN LIFE STAGES OF THE CADDIS FLY (*Hydropsyche cockerelli*) FROM THE COLUMBIA RIVER

Radionuclide	Adult to larvae radionuclide ratio $\frac{\text{radioactivity level in adults}}{\text{radioactivity level in larvae}}$
P^{32}	0.54
Sc^{46}	0.17
Cr^{51}	0.08
Co^{60}	0.34
Zn^{65}	0.15
As^{76}	0.048

The seasonal cycles of total beta emitters in *Hydropsyche cockerelli* and their planktonic food are compared to radioactivity of the water and water temperature in Figure 1. The concentration of radionuclides in the plankton, which consists mainly of diatoms and other algal forms, follows essentially the same seasonal pattern as the radionuclide cycle in river water, and the radioactivity of the water is essentially a pattern of the river flow since the reactor effluent is relatively constant. The radioactivity in the insects, on the other hand, coincides closely to the water temperature pattern. This shows that the influence of temperature upon food intake and metabolic rate obscures the influence of seasonal difference in radionuclide content of the plankton. Treherne (1954) found that the sodium-24 uptake rate by mosquito larvae was raised by increasing the temperature from 20° to 28° and 35°C.

The uptake rate of certain radionuclides by aquatic insects may be very rapid. This was observed by measuring the uptake of total beta emitters (dominantly phosphorus-32) by starved *Hydropsyche cockerelli* larvae when placed in cooled reactor effluent water and provided an alga grown in the same kind of effluent water (Figure 2). The larvae were collected from the Columbia River upstream from all reactor outfalls and were starved for 2 weeks prior to exposure to the effluent water. The alga *(Spirogyra)* was grown in the contaminated water until it reached

Table 4. RADIONUCLIDE CONTENT OF CADDIS FLY LARVAE *(Hydropsyche cockerelli)* FROM COLUMBIA RIVER DURING SUMMER AND WINTER

Radionuclide	pc/g wet wt.	
	February	August
Na^{24}	120	710
P^{32}	4200	24000
Sc^{46}	56	71
Cr^{51}	860	6000
Mn^{54}	100	79
Fe^{59}	19	
Co^{60}	30	2
Cu^{64}	310	7100
Zn^{65}	730	2000
As^{76}	150	520
Zr^{95}-Nb^{95}		66
Ru^{103}		110
Ba^{140}		42
La^{140}	170	350
Np^{239}		310

a radioactivity plateau (16 namocuries/g wet weight), and cut into short lengths to improve availability to the larvae. Comparable results were reported by Whittaker (1961) from a study of P^{32} movement in an experimental outdoor pond. He found an initial algae-to-mayfly nymph transfer rate (P^{32} concentration in mayflies/P^{32} concentration in algae) of 0.01 per hour. Whittaker's data show that the mayfly nymphs did not reach peak radioactivity until the fourth day; however, in his experiment, the isotope was introduced into an established ecosystem so that the algae did not reach peak radioactivity until the fourth day.

Much remains to be learned about the influence of water chemistry upon radionuclide uptake by aquatic insects and other organisms. Treherne (1954) found that raising the sodium content of water from 4 to 8 milliequivalents per liter or the potassium content from 0.159 to 4.00 milliequivalents per liter did not change the rate of sodium-24 uptake by mosquito larvae.

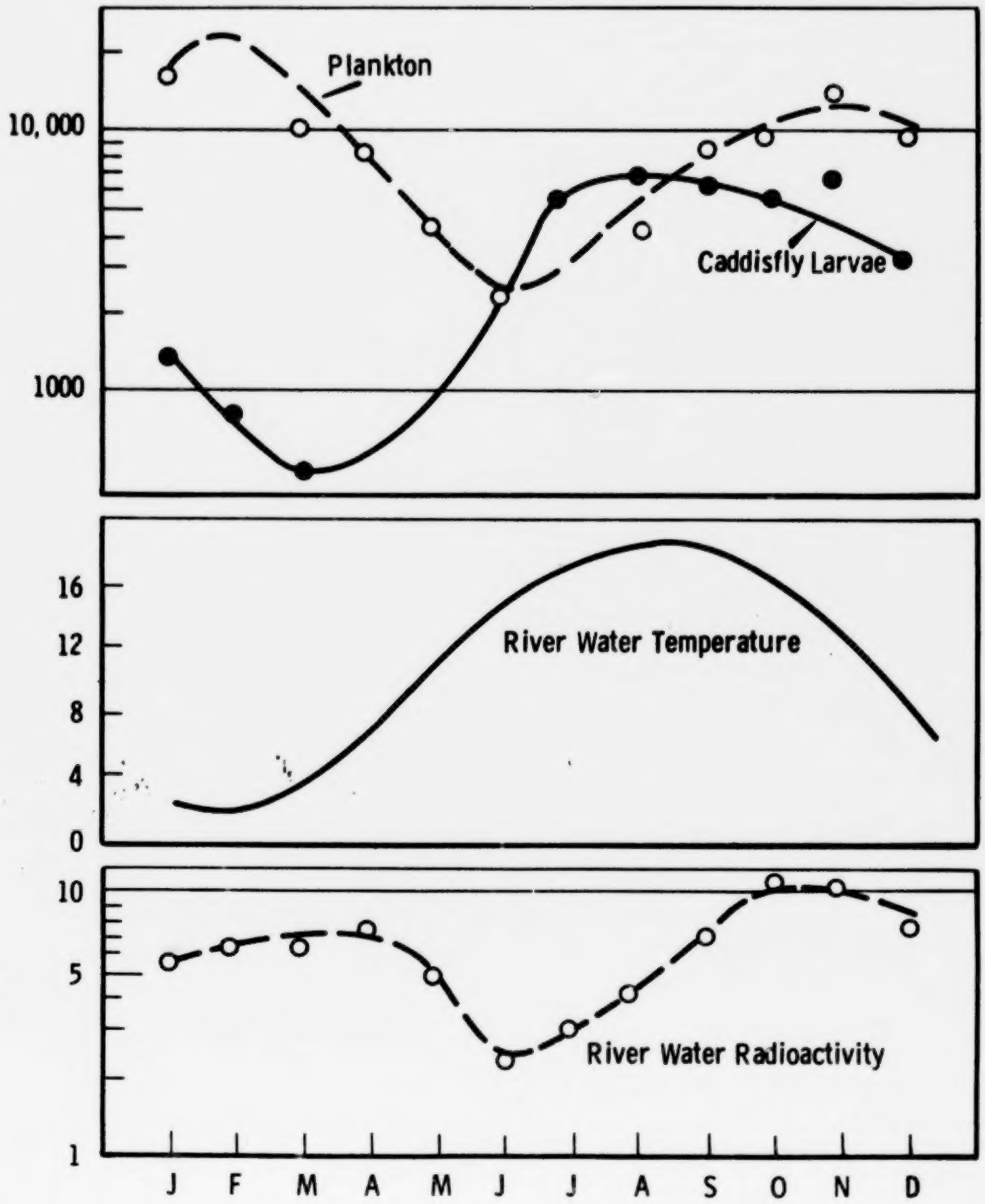

Figure 1. Beta-emitter concentration in caddis fly larvae *(Hydropsyche cockerelli)* and plankton from Columbia River.

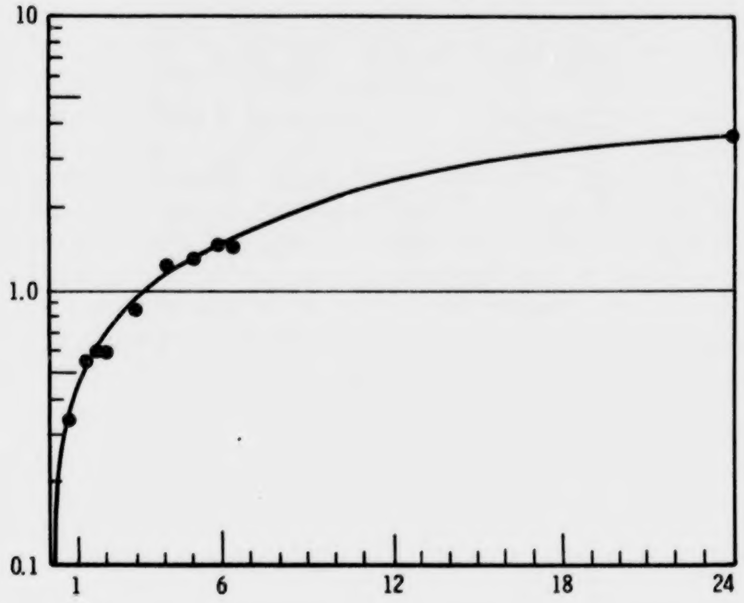

Figure 2. Uptake of beta emitters from reactor effluent water by caddis fly larvae *(Hydropsyche cockerelli)*.

I have investigated the influence of isotopic dilution upon phosphorus-32 accumulation in aquatic organisms by introducing the same trace amount of phosphorus-32 but differing quantities of stable phosphorus into each of four troughs containing established stream-type ecosystems. The biotic community was obtained by placing cobblestones supporting algae and invertebrates, *Elodea*, snails *(Radix)*, and juvenile suckers *(Catostomus)* into the indoor troughs of flowing water. The phosphorus-32 was metered into a common head tank of raw river water, which was discharged into each trough at the rate of 0.1 microcurie per minute. The levels of stable phosphorus tested in the troughs were 0.01, 0.056, 0.22, and 1.01 ppm. These were maintained by continuous metered addition of dilute phosphoric acid. Water temperature was maintained constant at 13°C. After 32 days of continuous administration of stable and radioactive phosphorus, the experiment was terminated and the biota analyzed for phosphorus-32.

The levels found in caddis fly larvae, fish (entire minus gut content), and sessile diatoms are shown in Figure 3. Similar patterns occurred for other organisms such as *Potamogeton*, *Elodea*, and snails. These results indicate a nearly linear reduction of phosphorus-32 uptake with increase of phosphorus in the water.

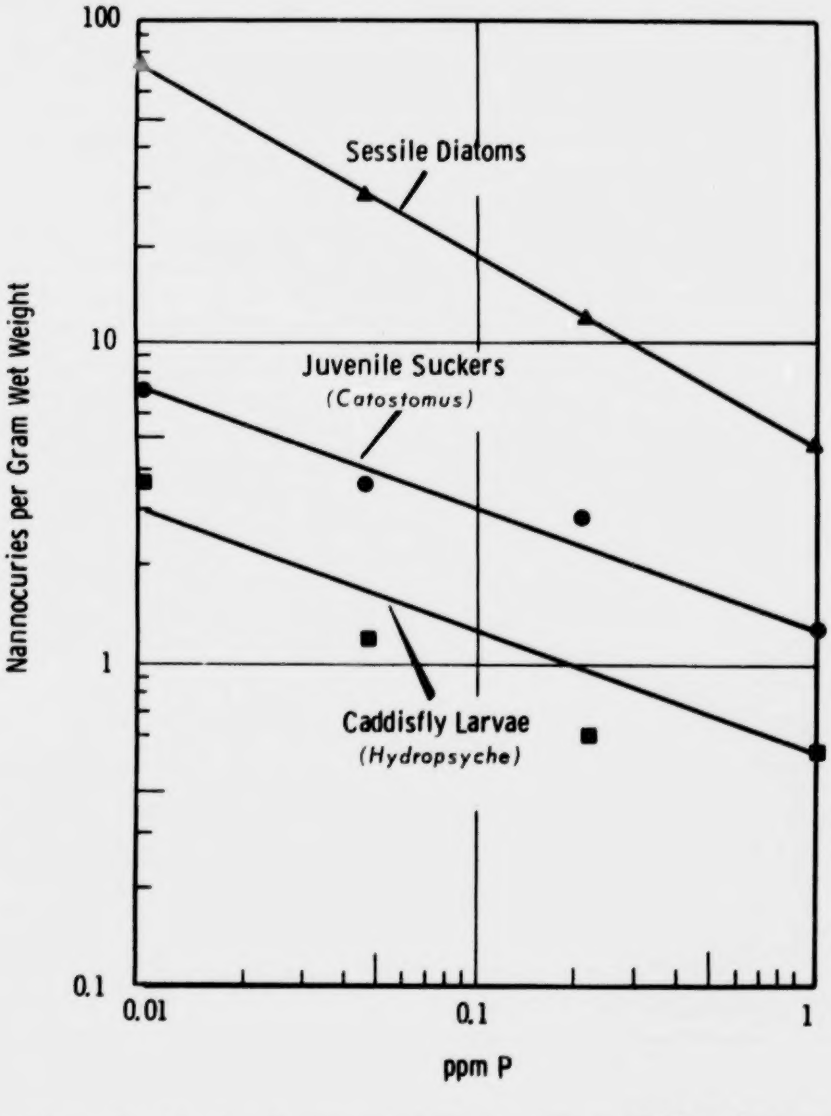

Figure 3. Effect of stable phosphorus concentration in water on P^{32} uptake by caddis fly larvae *(Hydropsyche cockerelli)*, fish, and sessile diatoms.

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RELATIONSHIPS BETWEEN THE CONCENTRATION OF RADIONUCLIDES IN COLUMBIA RIVER WATER AND FISH

R. F. Foster and Dan McConnon[*]

INTRODUCTION

The possibility that fish might pick up radionuclides from water containing radioactive wastes was recognized during the conceptional stage of the atomic energy era. This early recognition was prompted by considerations of the several ways by which people might be exposed to radioactive liquid effluents in the vicinity of the major atomic energy installations and the possible effect of radioactive effluents on valuable aquatic resources. The work of Prosser, *et al.* (1945) on the accumulation of fission products in goldfish, was probably the first work in this specific field that was motivated by radiological considerations; it was soon followed by laboratory programs at the University of Washington (Welander, 1945), and at the Hanford site (Foster, 1946). During the last decade many additional programs concerned with the accumulation of radioactive materials in fresh water and marine forms have become well established in the biological laboratories of universities and governmental agencies throughout the world.

Much of the current experimental work on radionuclides in fish continues to be stimulated by the potential effect of the radionuclides on the fish or the exposure of people who may eat them. Funds are justified, at least partially, on this basis. The cause remains a worthy one since actual field experience at major atomic energy sites has usually shown that the accumulation of one or a few radionuclides by aquatic forms that are eaten in quantity by man proves to be the factor which limits the quantity of radioactive material that may be released to surface waters.[†]

After some 20 years of recognizing the problem we have learned something about the mechanisms of uptake and the turnover rates for a few radionuclides in fish. We have also speculated a great deal about "concentration factors" and their importance in predicting allowable rates of release of radionuclides. However, when we are faced with new practical problems of applying concentration factors in order to estimate the level of specific nuclides that may result in fish as a consequence of known amounts in the water, we find that the best available information is rarely the product of controlled laboratory experimentation. Rather, the most usable information stems from field surveys made near the major atomic energy installations or in the Pacific Test area. The conclusion is obvious - we need more experiments designed to simulate closely field conditions and also greater exploitation of the data that can be derived from existing conditions. This paper is intended as a contribution in the second category and utilizes recent data obtained from the environmental surveillance program of the Columbia River downstream from the Hanford plutonium producing reactors. Data of a somewhat similar nature obtained in previous years have been presented on several occasions (Foster and Davis, 1955; Davis, *et al.*, 1958).

RADIONUCLIDES IN COLUMBIA RIVER WATER

Cooling of the Hanford reactors by direct use of filtered Columbia River water in a single pass system results in the formation of different neutron activation products, which enter the Columbia River with the effluent water. For the most part, these are not the same radionuclides that characterize world-wide fallout because they are not fission products. Relatively small amounts of fission products are also present, however, because of the fissioning of natural uranium present in the river water, because of occasional ruptures of the jackets of the fuel elements, and because of fallout. A description of the characteristics of the reactor effluent and how it is handled has been reported previously (Foster, *et al.*, 1961).

Before dilution with the main flow of the Columbia River, more than 60 different radionuclides can be identified in the reactor effluent. After dilution and transport by the river some 40 miles downstream from the reactors, about a dozen of the nuclides can still be measured by routine laboratory methods with reasonable success. Figure 1 shows the relative abundance of the dominant radionuclides at three points below the reactors: Hanford Ferry (5 to 20 miles below reactor outfalls), Pasco (35 to 50 miles below the outfalls), and Vancouver (about 260 miles below the outfalls). The relative concentrations of the several radionuclides shown changes rapidly with time (and distance) principally because of radioactive decay of the shorter-lived nuclides. For completeness, a list of all the radionuclides that have been identified in the reactor effluent is provided in Table 1.

The concentrations of radionuclides in the river fluctuate widely between seasons. This is partly because of variations in the physical and chemical characteristics of the river water and thus the material available for activation in the reactor, but the dominant factor is river flow which is low (about 50,000 cfs) in midwinter and high (400,000 to 500,000 cfs at the peak) during the spring freshet. Figure 2 shows the concentrations of the seven most abundant radionuclides in the Columbia River at Pasco for 1961 and the first quarter of 1962. The material presented in the next section will show that the concentration of radionuclides in the fish bears very little resemblance to that shown in Figure 2.

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[*] Radiation Protection Operation, Hanford Laboratories Operation, General Electric Company, Richland, Washington.
(Work performed under Contract No. AT(45-1)-1350 between the U. S. Atomic Energy Commission and the General Electric Company).

[†] Current estimates of the fraction of permissible radiation exposures that can result from the use of Columbia River water and fish downstream from the Hanford plant are reported by Nelson (1962).

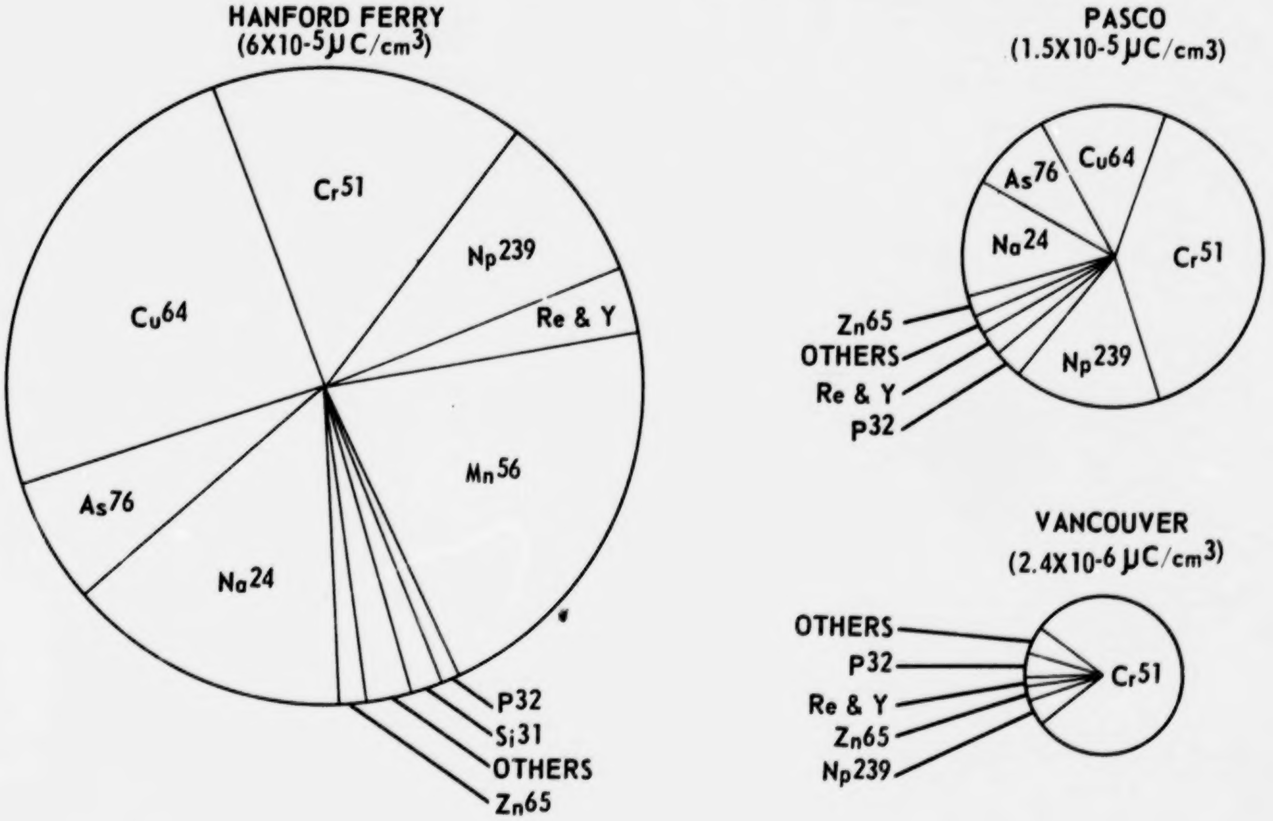

Figure 1. Relative abundance of radionuclides in Columbia River water.

Table 1. RELATIVE ABUNDANCE OF REACTOR EFFLUENT RADIONUCLIDES
(Reference Time - 4 Hours Postirradiation)

Major, 90%	Minor, 8%	Trace, 2%		
Mn^{56}	Zn^{69}	Eu^{152}	I^{131}	Pr^{145}
Cu^{64}	Ga^{72}	Sm^{153}	Ce^{141}	Pm^{151}
Na^{24}	Sr^{92}	W^{187}	Pr^{142}	Co^{60}
Cr^{51}	U^{239}	La^{141}	C^{14}	Pr^{143}
Np^{239}	I^{133}	Nd^{149}	Nd^{147}	Ru^{103}
As^{76}	Y^{92}	La^{140}	Ca^{45}	Sc^{47}
Si^{31}	Nb^{97}	I^{132}	Ag^{111}	Sr^{90}
	Sr^{91}	Eu^{157}	Y^{91}	Cs^{137}
	Zn^{65}	Ba^{140}	Fe^{59}	Sr^{85}
	P^{32}	Mo^{99}	Sr^{89}	U^{238}
	Y^{90}	Sm^{156}	Mn^{54}	Pu^{239}
	I^{135}	Sc^{46}	Zr^{95}	Ac^{227}
	Y^{93}	Cd^{115}	Pm^{149}	Po^{210}
		Ce^{143}	Eu^{156}	Tb^{160}
		Pm^{147}	$Ce\text{-}Pr^{144}$	Co^{58}

RADIONUCLIDES IN COLUMBIA RIVER FISH

Some radioactive materials can be detected in all species of fish (and other aquatic life, for that matter) in the Columbia River (Olson and Foster, 1952). There are, however, significant differences in the quantities of particular radionuclides present in different species (Davis, *et al.*, 1958). A number of reasons for this can be advanced (Davis and Foster, 1958), but two of the most obvious are food habits and migratory habits.

In order to clarify the relationship between the concentration of radionuclides in the water and in the fish it is desirable to avoid the introduction of unnecessary variables. For this reason, this discussion is confined to a single game species of fish, the Rocky Mountain whitefish *(Prosopium williamsoni)*, sampled from a 20-mile stretch of the Columbia River (Hanford Ferry to Richland); only the muscle tissue of adult specimens is included. Figure 3 shows an approximation of the radionuclide content of the muscle of such specimens collected during the fall of 1961, the time of the year when the highest concentrations are observed. The phosphorus-32 and zinc-65 clearly contribute the greater part of the radioactivity. For comparison, the radionuclide content of the Columbia River water at the same season and location is also presented in Figure 3. The differences between the relative amounts of radionuclides in the water and in the fish are obvious. The areas of the circles in Figure 3 are proportional to the total radioactivity of the water and the fish they represent. Several of the nuclides that are relatively abundant in the water are not easily measured in the fish, and some nuclides readily detected in the fish, such as cobalt-58, cobalt-60 and naturally

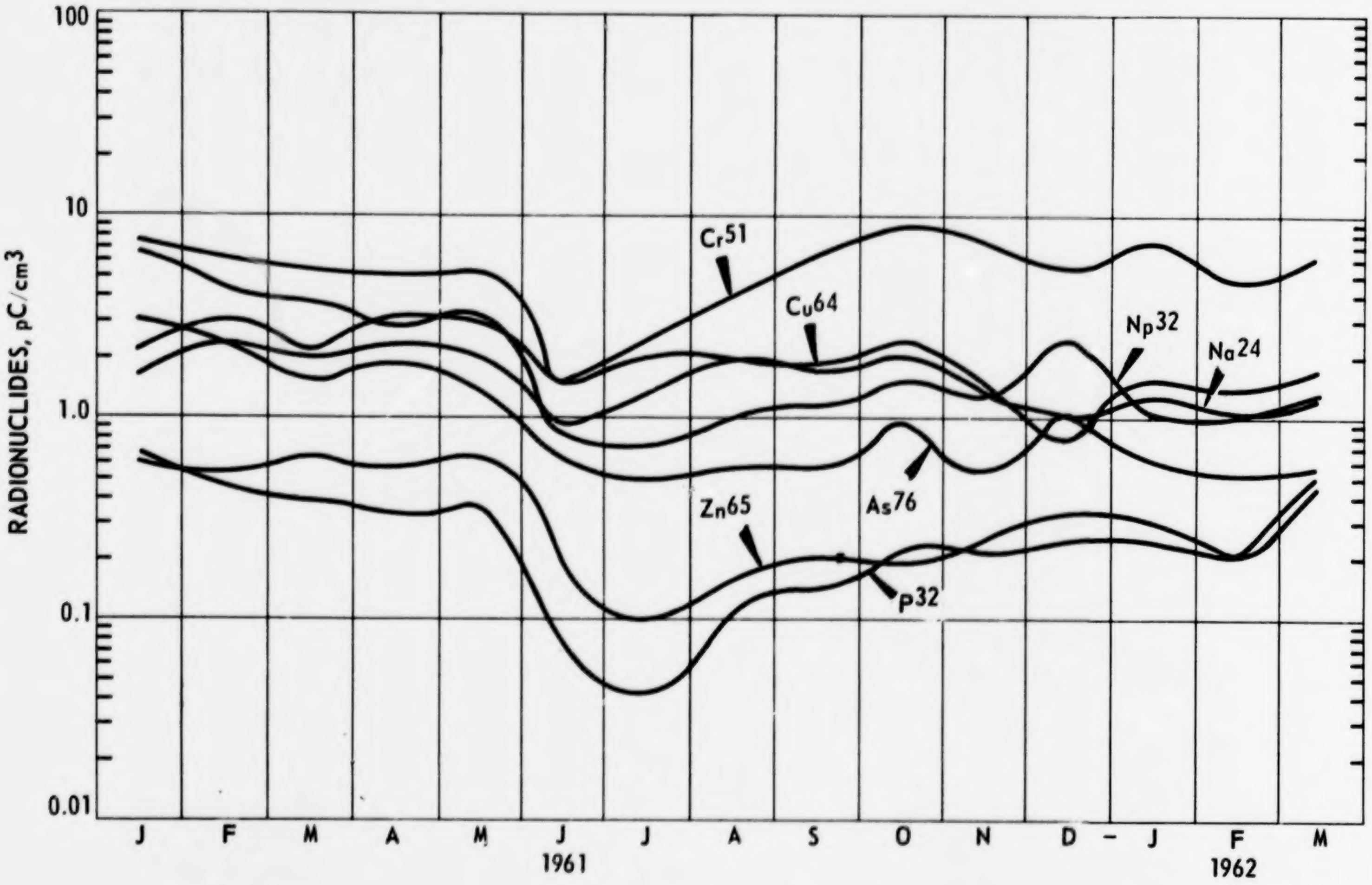

Figure 2. Concentrations of several radionuclides in Columbia River water at Pasco, Washington.

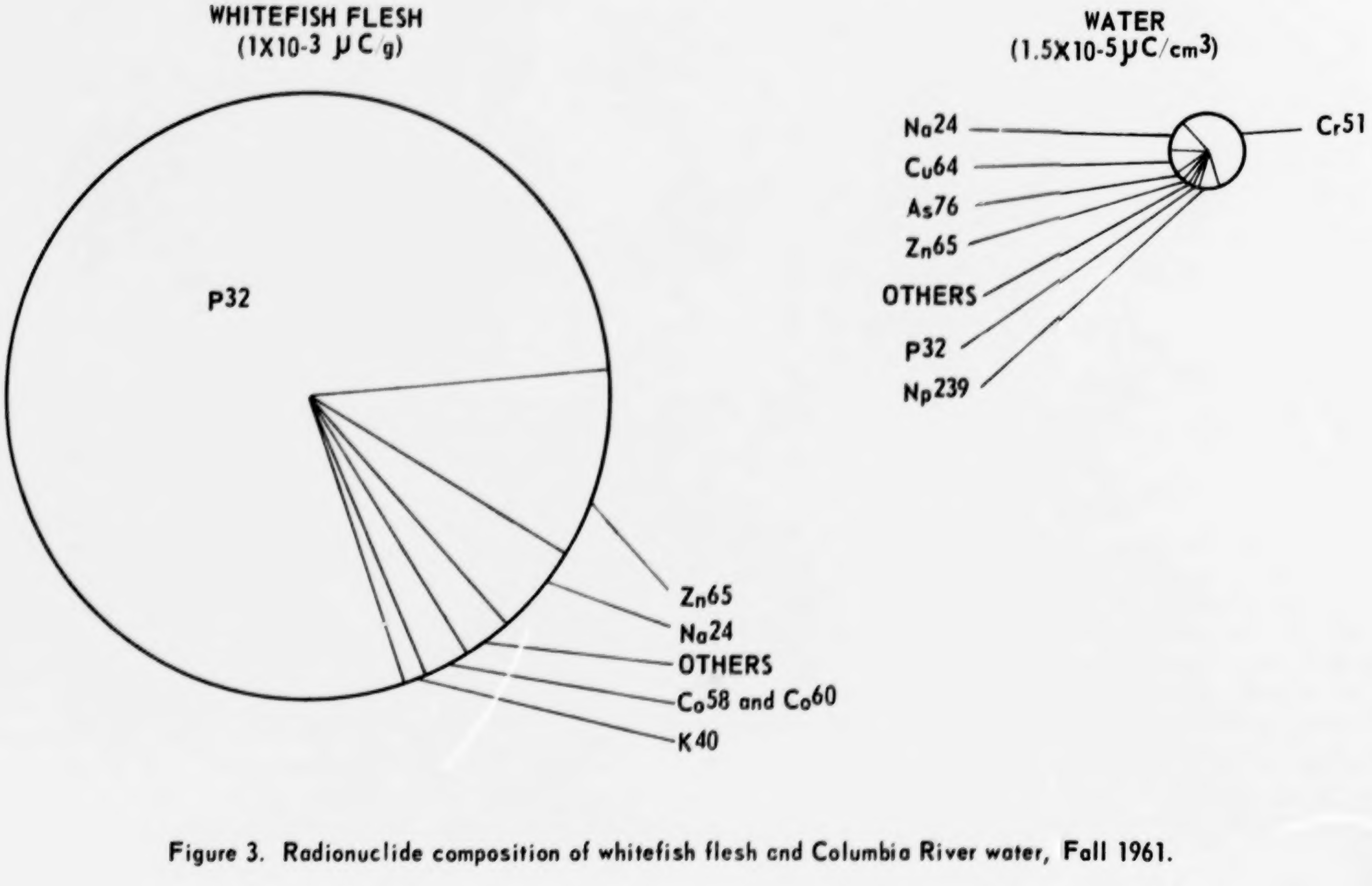

Figure 3. Radionuclide composition of whitefish flesh and Columbia River water, Fall 1961.

occurring potassium-40, are not easily measured in the water. It must be stressed that Figure 3 provides only an approximation of conditions that existed at a given time. The ratios actually change quite rapidly from week to week.

The wide variations encountered between specimens caught at the same time and place and at different seasons of the year are shown in Figures 4 and 5 for phosphorus-32 and zinc-65, respectively. As shown in Figure 4, the range in the concentration of phosphorus-32 in fish collected on a particular day commonly extended over an order of magnitude. On rare occasions the range was spread over two orders of magnitude. Within a year the difference between the most- and least-radioactive fish may approximate 1000. Such wide variations should discourage investigators from drawing sweeping conclusions on the basis of spot samples of a few fish taken once or twice a year. Data presented in Figure 4 are for one species of fish; inclusion of other species would serve to widen the variation still more. A contributing factor to the observed variation is the relatively short (2-week) half-life of phosphorus-32. This means, in effect, that the phosphorus-32 content of the fish reflects the fish's experience with this radionuclide over the previous 1 or 2 months. While an appreciable amount of integration of the intake and loss of phosphorus-32 takes place in this interval, it is still too short a time to smooth out fluctuations resulting from major changes in feeding rates and migrations and emigrations from the section of the river where the phosphorus-32 is most abundant.

Variations in the zinc-65 content of the fish, while still quite large, are not as great as for phosphorus-32. Figure 5 shows a seasonal fluctuation of about 10 fold, compared with about 50 fold for phosphorus-32 in the same fish. The radioactive half-life of zinc-65 is about 9 months; thus the zinc-65 concentration in the fish may reflect an integrated experience over a much longer time interval than for phosphorus-32. The biological turnover, however, of zinc-65 probably has a greater effect on the accumulated body burden than the radioactive half-life.

FACTORS AFFECTING THE PHOSPHORUS-32 CONTENT OF THE FISH

The two dominant modes by which radionuclides in the water reach the flesh of fish are quite obvious; one is direct absorption through the gills or other external surfaces into the blood stream, and the other is ingestion and assimilation with food. The relative importance of these two modes differs greatly with different elements and carefully controlled laboratory work is necessary for a satisfactory resolution. Nevertheless, field data, such as that shown in Figures 4 and 5, provide useful clues. The phosphorus-32 data have been selected for further analysis at this time because supporting information is more complete than for zinc-65.

The seasonal variations in the phosphorus-32 content of Columbia River fish (Figure 4) have been attributed principally to variations in the intake rate of phosphorus-32 with food organism. (Davis and

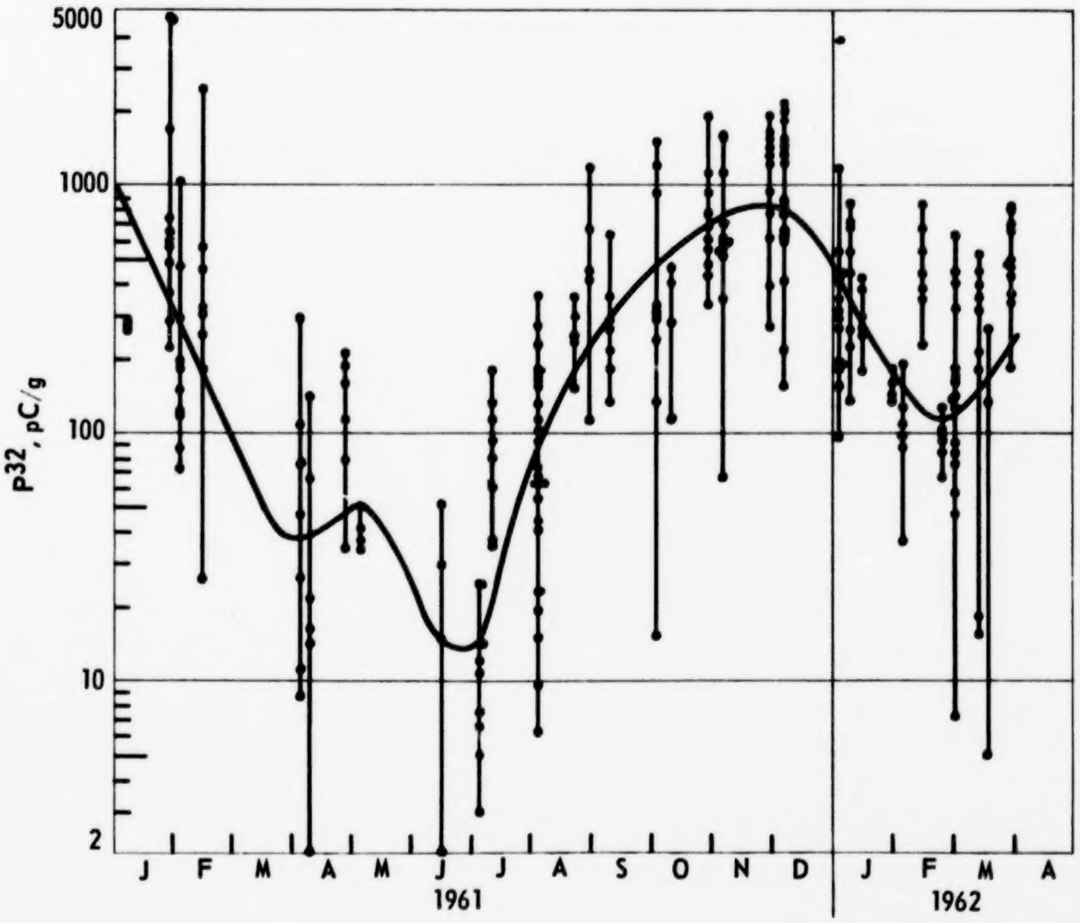

Figure 4. Concentration of P32 in the flesh of whitefish caught between Hanford Ferry and Richland.

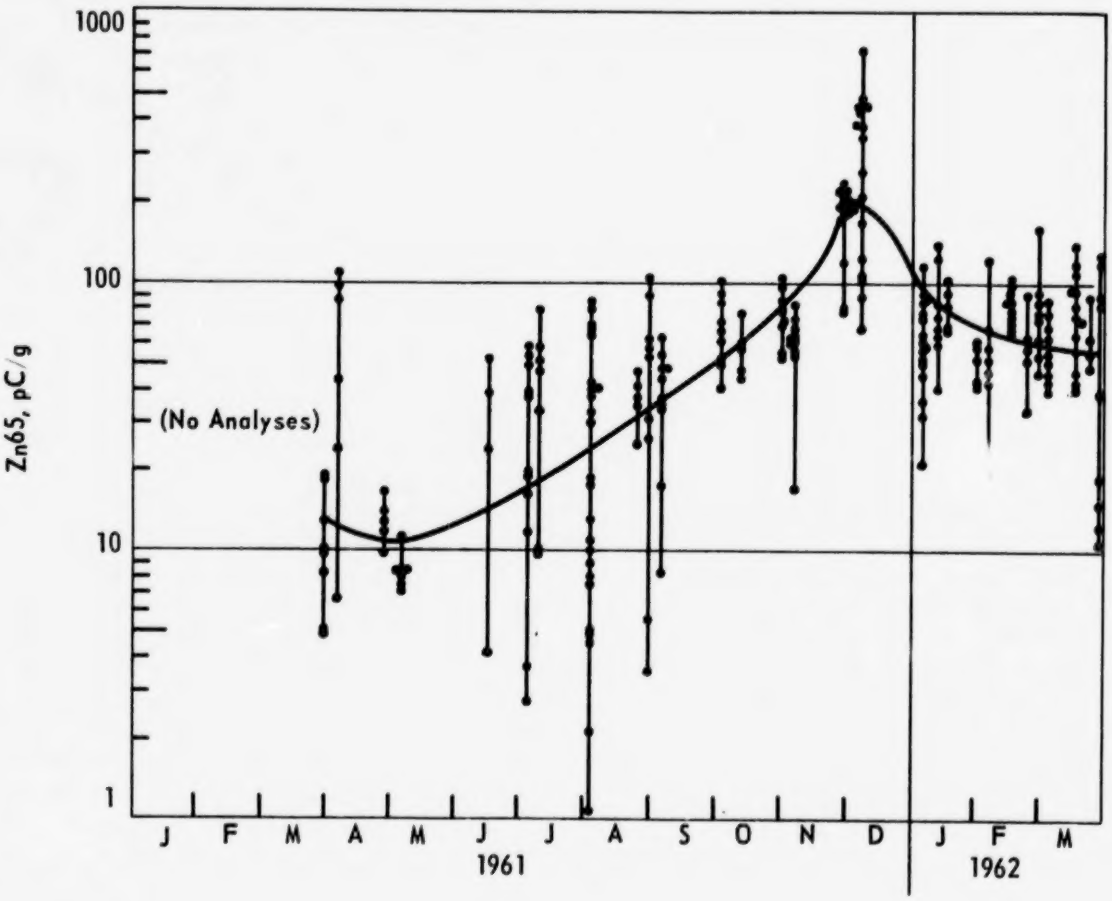

Figure 5. Concentration of Zn^{65} in the flesh of whitefish.

Foster, 1958). It should be recognized, then, that the availability of phosphorus-32 to the fish is greatly influenced by complex ecological relationships that involve all the components of the food base for the fish. While a detailed resolution of this problem would be most interesting, a first approximation of the water-fish relationship can be worked out even though knowledge of the food supply of the fish is incomplete. Such an approximation is attempted here.

Watson (1957) has shown that phosphorus-32 once deposited in fish diminishes very slowly - in effect, at about the rate of radioactive decay of the nuclide. Therefore, the amount of phosphorus-32 observed in Columbia River fish can be viewed as a consequence of two primary factors: one, the amount the fish contained a few days prior to sampling, which can be considered as a base; and two, the amount of new phosphorus-32 acquired during a few day's time, which is added to the base. For convenience, 1 week was selected as an appropriate period over which the rate of change or acquisition of new phosphorus-32 would be studied.

With the curve plotted in Figure 4 as a basis, the amount of new phosphorus-32 acquired by the fish each week throughout the 15-month period was computed (with proper allowance for radioactive decay). The result is plotted in Figure 6. Between January and mid-March, 1961, the fish acquired little to no new phosphorus-32 and the concentration in the flesh declined at essentially the radioactive decay rate of the nuclide. The fish took in a small quantity of phosphorus-32 in April of 1961, but it was not until July that the intake rate increased markedly. The maximum rate of intake occurred about the first of November, and thereafter it diminished rapidly.

Figures 4 and 6 do not take into account the fact that the concentration of phosphorus-32 in Columbia River water fluctuates with time. Obviously the amount of phosphorus-32 that can be acquired by the fish is dependent upon the amount available in the water, even though a food chain is operative as a vector. Figure 2 shows the measured concentrations of phosphorus-32 in the Columbia River water just downstream from the section of the river from which the whitefish were taken. The minimum values, which occurred in June and July, reflect a high discharge into the river. Lesser amounts of phosphorus-32 were introduced into the river during the last quarter of 1961 than during the first quarter (Figure 2). The effect of this fluctuating concentration of phosphorus-32 in the river water on the rate of uptake by the fish can largely be removed by adjusting the data plotted in Figure 6 to an assumed uniform concentration of one micromicrocurie of phosphorus-32 per cubic centimeter of water. Figure 7 shows the rate of uptake of phosphorus-32 by the fish (in terms of picocuries of phosphorus-32 per gram of fish per week) per picocuries of phosphorus-32 present in a cubic centimeter of river water. It should be remembered that this relationship is largely empirical. Although it excludes the role of the food chain, the shape of the curve emphasizes its existence.

The data in Figure 7 have been plotted in such a manner that the curve would be a horizontal line if there were a simple direct relationship (e.g. constant proportionality) between the concentration of

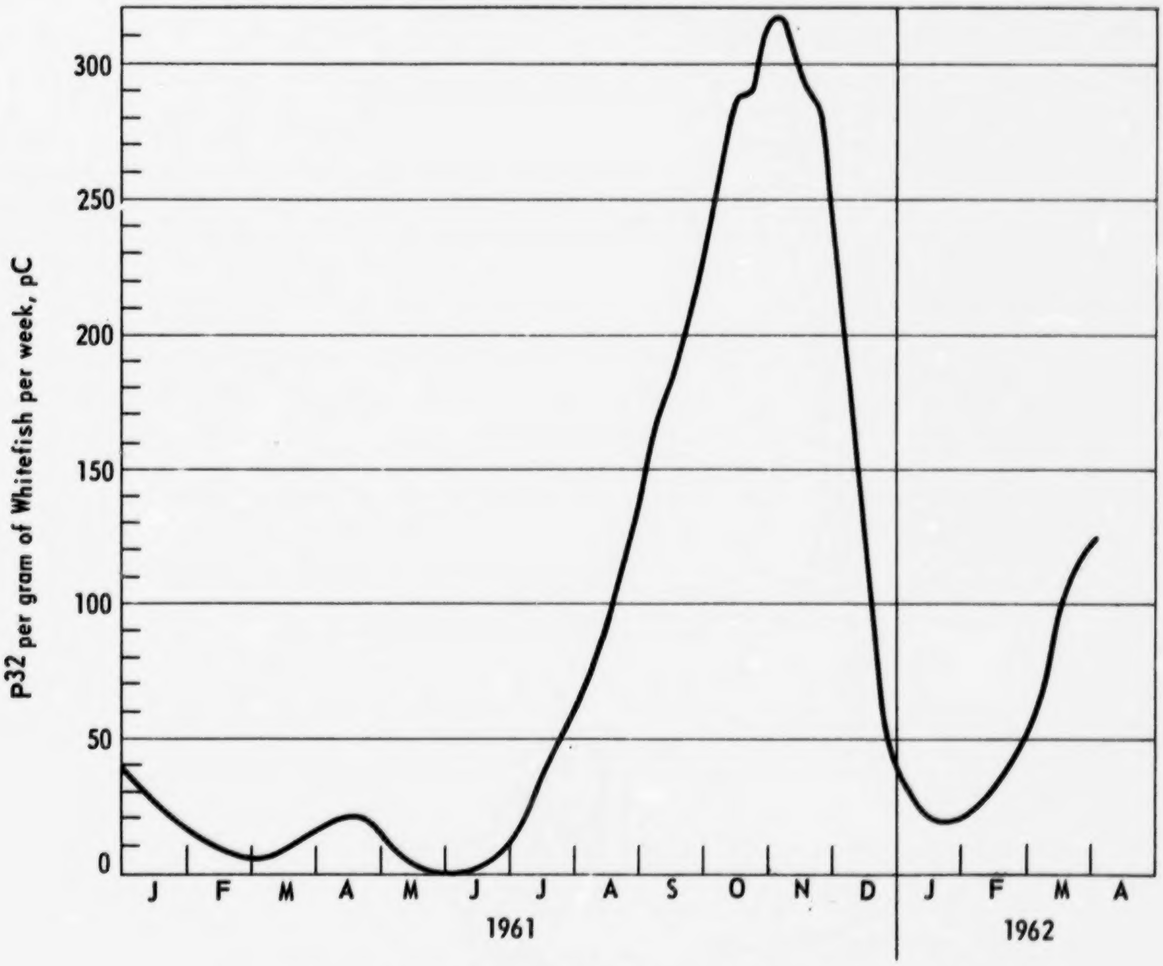

Figure 6. Rat at which new P^{32} was deposited in the flesh of whitefish.

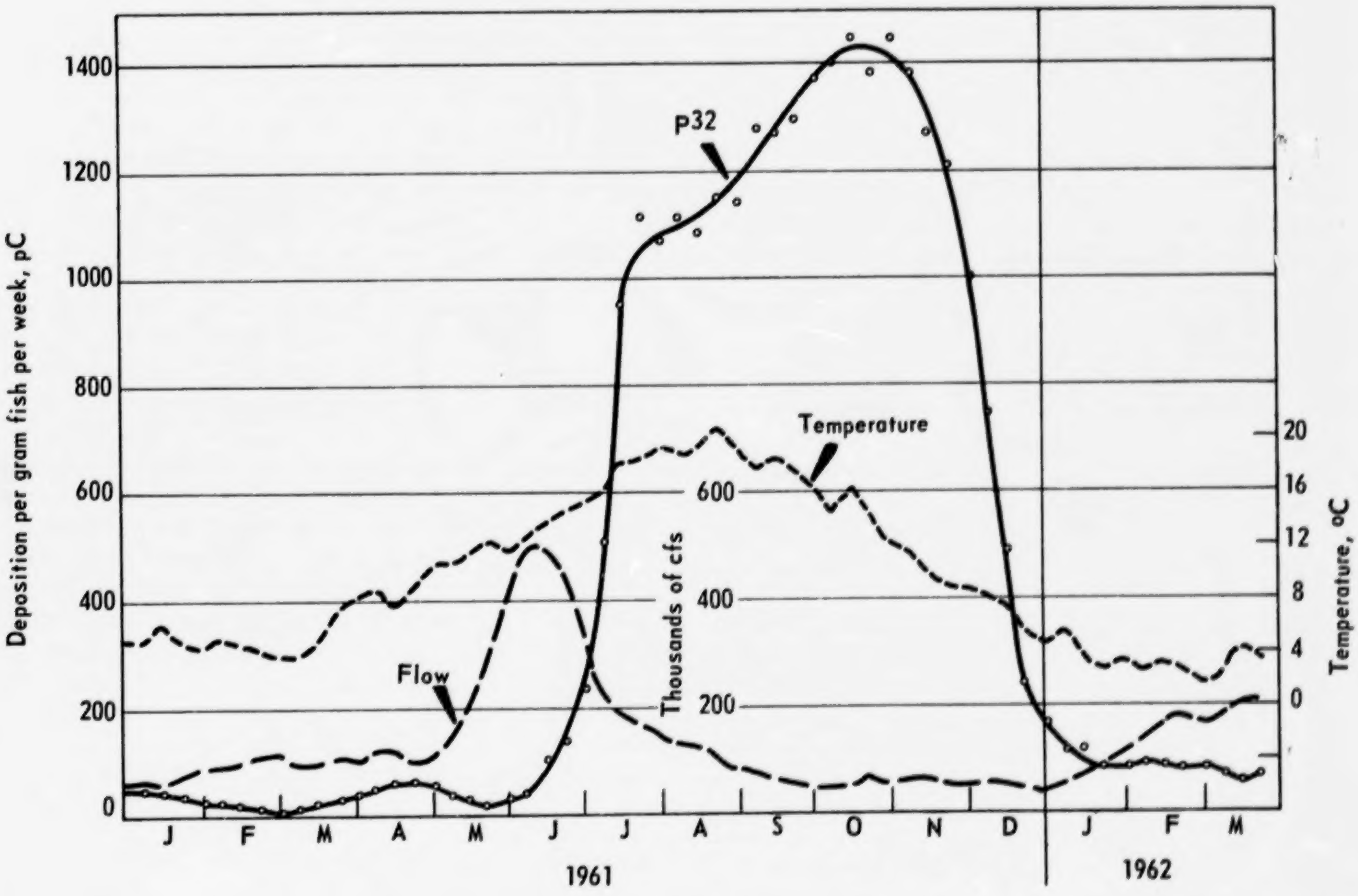

Figure 7. Deposition of P^{32} in fish related to river stage and temperature – rate per picocuries of P^{32} per cubic centimeter of water.

phosphorus-32 in the water and the quantity taken up by the fish per unit time. Since this is not the case, some interpretation is in order. It has been proposed that temperature, as a dominant factor affecting metabolic rate, strongly influences the rate of accumulation of radionuclides (Davis and Foster, 1958). In order to observe the temperature vs. phosphorus-32 accumulation relationship for the adult whitefish, the temperature of the Columbia River water has been plotted in Figure 7. The relationship is obviously not entirely consistent, but certain features are evident. At temperatures below 5°C uptake of phosphorus-32 is very slow, and the maximum rate of uptake occurs in the range of 12° to 16°C. It is not known whether the slower-than-maximum uptake, which occurred in August when the water was the warmest, resulted from temperatures above optimum for this species, from a less favorable food supply, or for some other reason. For comparable temperatures, the rate of uptake of phosphorus-32 was much greater in the fall than in the spring.

Since the stage of the river can have a heavy influence on the availability of food for the fish, a plot of the river discharge is also included in Figure 7. The usual high flow, caused by melting snow, occurred in May and June. This spring freshet is accompanied by high turbidity, shifting of the substrate, and increased velocities, all of which are detrimental to the food base. Re-establishment of an adequate food supply after the spring run-off could easily account for the rapid rise in uptake of phosphorus-32 by the whitefish during July. The phosphorus-32 curve in Figure 7 is unaffected by river flow in the sense that it affords dilution of the radioactive materials, since the data plotted in this figure have already been adjusted to a uniform concentration of phosphorus-32 in the water.

CONCENTRATION FACTORS

For the purpose of predicting the concentration of certain radionuclides in fish that results when there is continuous discharge of a radioactive effluent, it is convenient to apply "concentration factors" that express the expected level in the fish as some multiple of the level in the water. For common elements theoretical concentration factors can be derived from quantitative measurement by conventional chemical analysis of the stable isotopes in both the water and the fish. Field experience with radioisotopes has shown that the theoretical values are not usually reached.

The variable rate of accumulation of phosphorus-32 in whitefish flesh shown in Figure 7 indicates a similar seasonal variation in the concentration factor. The dimensions of Figure 7 are, of course, not quite those of the conventional concentration factor (microcuries per gram of fish in relation to microcuries per cubic centimeter of water), but they differ only by a constant needed to convert the rate of uptake (microcuries of phosphorus-32 per gram per week) to the ultimate equilibrium level that this rate would yield. On the basis that phosphorus-32 is depleted from the fish only by radioactive decay with a half-life of 2 weeks, the needed correction factor is 3.5. The concentration factor for the flesh of adult whitefish under conditions prevailing in the Columbia River can, then, be stated as ranging from essentially zero during the coldest period of the year to about 5,000 in October. The maximum of 5,000 would only be observed, however, if the maximum rate of uptake (1400 picocuries of phosphorus-32 per gram per week) continues for nearly a month in order for an equilibrium to be reached. For all practical purposes this did occur in 1961.

The rate of uptake curve shown in Figure 7 has another interesting feature related to concentration factors. The fact that there is no well-defined plateau associated with the maximum values suggests that appreciably different peaks are likely to occur in other years when ecological conditions might be more or less favorable. Thus, the concentration factor of 5,000 calculated for 1961 is not necessarily applicable to other years.

A more direct and less sophisticated method of arriving at the maximum concentration factor for phosphorus-32 is merely to take the maximum observed concentration of phosphorus-32 in the fish and divide it by the concentration observed in the water at about the same time. In this case, one should not merely use the concentration in the water at the time the fish were sampled, but rather a value which is more representative of conditions over the interval of time during which the fish acquired its burden of the radionuclide. For example, if the values shown in Figure 4 (900 picocuries of phosphorus-32 per gram of fish in late November) and Figure 2 (0.23 picocuries of phosphorus-32 per cubic centimeter of water) are used, a concentration factor of about 4,000 is obtained.

Concentration factors for zinc-65 cannot at this time be derived on a basis of intake rates because adequate data on turnover of zinc-65 in fresh water fish are not available. Values can, however, be derived on the basis of the ratio of the concentration of zinc-65 in the water to that in the fish. With the zinc-65 concentrations in whitefish flesh shown in Figure 5 and the concentrations in the water shown in Figure 2, a maximum concentration factor of about 1000 is obtained; this occurs in November.

Concentration factors for the other radionuclides often observed in the whitefish flesh can be stated only in vague terms because of the difficulty in measuring them at their relatively low levels in samples processed on a "production-line" basis. The half-life of sodium-24 is so short (15 hours) that any appreciable delay in processing samples requires the application of large correction factors. The short half-life also greatly limits the fraction of this nuclide that can enter the fish via complex food chains. On the basis of the limited data available at this time, however, the concentration factor for sodium-24 between water and whitefish flesh appears to be in the range of 100 to 500.

Cobalt-58 and -60 constitute an interesting case since the level of these nuclides in the river water is below the detection level of the laboratory techniques used for routine samples. Extrapolation from limited data on the rate of release of these nuclides into the river would, however, place the order of magnitude of the concentration factor at 10,000.

Of the more abundant radionuclides in the water (see Figure 3), copper-64, and arsenic-76 have such short half-lives (13 hours and 26 hours, respectively) and limited direct uptake from the water that they are not readily detected in fish samples processed by routine methods. Available data indicate that the concentration factors for these nuclides are something less than 10.

The half-lives of chromium-51 and neptunium-239 are 28 days and 2.3 days, respectively, but these elements do not appear to be taken up in significant amounts directly from the water nor are they accumulated by food organisms (Foster, 1961). The failure of routine laboratory analyses to detect these radionuclides in the whitefish indicates that they can have concentration factors of no greater than 10.

CONCLUSIONS

The uptake of radionuclides by fish is a function of a number of interacting factors, which results in wide variability. This variability is so great that a large number of samples collected over at least one year's time is necessary in order to determine the average concentration in the fish and fluctuations with time - at least in the case of radionuclides with relatively short effective half-lives.*

Concentration factors derived on a theoretical basis, which assume a high rate of intake by the fish throughout the year, will substantially overestimate the quantities of radionuclides that will accumulate in the fish. This generalization should hold for radionuclides with effective half-lives of several weeks or less in ecological conditions subject to wide variations between seasons.

ACKNOWLEDGMENTS

The fish used in this study were collected by members of the Environmental Monitoring group under conditions that often required special skill and effort. The success of any project of this nature is heavily dependent upon the quality and timeliness of the radiochemical analyses performed on the samples brought in from the field. We are deeply grateful to Mr. Fred Holt and the members of his Radiological Analysis Operation, and especially to Mr. Dave Argyle, for providing this service. Mrs. Darlene Moore made a very substantial contribution to this paper by organizing the data and converting it into the useful forms presented. We also appreciate the significant effort applied to the analysis of this data by Mr. Ray Buschbom of the Operations Research and Synthesis Operation.

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* Effective half-life refers to the time required for a radionuclide deposited in a tissue to be dimished by one-half as a result of the combined action of radioactive decay and biological elimination.

DISCUSSION

Dr. Oscar Ravera supplied the following detail on his paper: the level of radioactivity was determined in 50 liters of filtered water. At the present time only few data on the radioactivity of sediments in Lake Maggiore are available.

In the discussion it was mentioned that an increase in beta activity with depth was also found in Lake Michigan.

Concerning the disposition of radioactive wastes in the sea, it was suggested that deposition sites should be greater than 2500 feet deep to prevent our food organisms from having direct access to them. This suggestion does not advocate, however, unlimited disposal of radioactive wastes in the sea, even though the thermocline acts as a barrier to the distribution of radioactivity introduced.

As an example of distribution of radioactivity, some data obtained in connection with the testing of weapons in the 1958 series were reported: 2 days after contamination of the area, 50 percent of the radioactivity in the plankton was tungsten-185, and 50 percent of that was associated with the silica in the plankton, mainly in diatoms. Examination of the same sample of plankton 3 weeks later showed a very high tungsten-185 content of the water, but the plankton was practically free of this nuclide.

It was stressed that the age and physiological state of the organism are most important for comparative studies of uptake of radioactive elements.

One explanation of the mechanism of uptake of radioactive elements was that the early uptake is almost 100 percent surface adsorption, i.e., physical in nature, and that later selective uptake and intake play the dominant role.

The isotope dilution concept, or specific activity approach, may not be applied to fresh water as it is applied to sea water; the results are consistent only in the latter.

It was pointed out that the uptake of phosphorus-32 and probably other nuclides by live algae is many times higher than the uptake by dead algae. Uptake is also different, and often manyfold higher, under natural conditions than in cultures. Thus, only ecological studies picture the real uptake.

Radionuclides may be returned to the water when the organisms containing them are killed.

The potential effect on people of radioactive uptake by algae blooms appears to be small and of short duration. Fishes, not algae, are a large potential source of radioactive material in the diet of people, unless one drinks unfiltered raw water.

When the effects of strontium-90 or cesium-137 are evaluated, specific activity criteria should be used rather than measurements of absolute activities.

The level of radionuclides, especially phosphorus, in the blood of fish was not determined in Dr. Foster's work, but he stated that such determinations have been made by some of his colleagues.

INFORMAL DISCUSSION SESSIONS

BIOLOGICAL INDICATORS OF POLLUTION

ALGAE AS INDICATORS OF POLLUTION

*Ruth Patrick**

Since the beginning of the twentieth century there have been numerous publications on algae as indicators of pollution. In this paper I will refer only to some of the literature that has described various methods or approaches in using algae for this purpose. In the early part of the century most workers used the word "pollution" to refer to the presence of an organic load, which mainly resulted from the entrance of sanitary wastes into a river. Since this type of pollution generally contained similar types of chemical compounds it was reasonable to suppose that one might discover species that would indicate various stages in the assimilation of these wastes by a river.

One of the most important, if not the most important, papers concerned with species indicating stages of pollution was the one of Kolkwitz and Marsson (1908). They classified organisms in organically polluted rivers according to the condition of the river area in which they were found. Five zones or conditions of a river's organic load were recognized. The polysaprobic zone was characterized by a wealth of high-molecular, decomposable organic matter. Chemicals were usually present in a reduced state and little if any dissolved oxygen was present. The α-mesosaprobic zone represented the stage in the recovery of a river from heavy organic pollution in which complex organic matter was present but oxidation was proceeding. The β-mesosaprobic zone was that in which most of the organic matter had been mineralized. The oligosaprobic zone was the zone of cleaner water in which mineralization had been completed. The katarobic zone was that of the clean, unpolluted water often found in mountain streams. Each of these zones or conditions contains species belonging to all major groups of algae found in fresh water, the bluegreens, greens, and diatoms. The presence of the species belonging to a given condition, for example "α-mesosaprobic," indicated that this condition existed. Many workers such as Hentschel (1925), Naumann (1925, 1932), Butcher (1947), and Liebmann (1951) used this system. They became aware that species classified as characteristic of a zone of pollution often did not occur when such a condition was present, or might be found under very different river conditions (Liebmann 1942, Thomas 1944).

This lack of applicability was due to several things. One was the realization, as Butcher (1947) stated, that species of algae are often resistant to pollution rather than grow well because of substances found in pollution. Thus these species may be found in many other river conditions besides those to which they were assigned by Kolkwitz and Marsson. Another important reason why their system failed was that pollution had changed so much since the early part of the century. It then mainly referred to an organic load of a particular type; today it is a collective noun referring to conditions resulting from the inflow of many kinds of chemicals, some of which are very toxic; various physical conditions such as hot water; and various kinds of organic materials coming from many industrial sources as well as sanitary wastes.

Thus, owing to the complexity of pollution, it became more and more difficult to state that any one species might characteristically be found in all kinds of pollution or be generally indicative of pollution. Wuhrmann (1951), Sramek-Husek (1956), and Fjerdingstad (1960) increased the number of zones characteristic of stages of pollution from the 5 recognized by Kolkwitz and Marsson to a total of 15 recognized by Wuhrmann.

Thienemann (1939) was the first to emphasize the fact that certain groups or associations of species (biocoensis) were characteristic of a given type of environment or biotope.

He gave the name "coenobionts" to those species composing an association of species characteristic of a single kind of biotope. Furthermore, the species characterizing the association were always found in large numbers.

"Coenophile" species were those species that had their best development in a given biotope but might be found as part of the biocoensis of other biotopes in which they were represented by smaller populations.

"Coenoxene" species were those species that did not seem to be characteristically associated with any particular biotope but were found in small numbers in many different biotopes.

Although Thienemann recognized the importance of the association of species in characterizing ecological conditions, he did not apply this system to the algae by listing species belonging to these various groups.

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Schroeder (1939), Häyren (1944), and Butcher (1947) were among the first to identify algae associations with varying degrees of pollution. Fjerdingstad (1950) recognized in algae communities dominant species, associate species, and accidental species and classified these according to the Kolkwitz and Marsson system. He classified the various species found by determining if they were dominant, associates, or accidental occurrences in the various zones or stages of pollution. Hustedt (1957) listed species that indicate heavy pollution if present in large masses.

Patrick (1949) further emphasized the importance of considering the structure of the communities of aquatic organisms including algae. In these mesotrophic to eutrophic streams, she considered as important the kinds of species, the sizes of their populations, and the numbers of species composing the algal communities. Those algal communities characteristic of natural or "healthy" areas of streams in the Conestoga Basin were characterized by a large number of species, most of which were represented by relatively small populations. These numbers of species did not change much from season to season in the same area so long as the ecological conditions were normal or natural. Furthermore, in 1961 Patrick stated that in similar ecological areas of streams in the same general geographical areas the numbers of species did not vary greatly. One of the first signs of pollution was the elimination of some of the more sensitive species and the development of large populations by some of the more tolerant species.

Patrick, in her various studies, has produced a great deal of data that support the principles set forth by Thienemann in 1920 and 1939, that is, that the optimum areas are characterized by a large number of species with relatively small populations.

In more recent studies the general emphasis has shifted from the importance of the mere presence of certain so-called indicator species to the consideration of the whole structure of the algal community, that is, the kinds of species, their relative abundance in the community, and the total number of species present.

Patrick et al. (1954, 1956) working with the structure of diatom populations came to the conclusion that the structure of a natural diatom community might be correctly represented by a normal curve (Figure 1). The shape of the curve did not change greatly so long as the environment did not vary more than one would expect from changes in the natural environment (Table 1). If the area under consideration became polluted, the shape of the curve would change depending on the amount of pollution. If the pollution was organic in nature and not very severe, certain species would be represented by very large populations and thus become much more dominant (Figure 2). However, this same effect might result from the presence of other types of pollution that do not have a very toxic effect. Under extreme conditions resulting from many different types of pollution, not only do many species die, which reduces the species number, but those that remain may have very variable sizes of populations depending on their ability to resist the particular pollutant (Figure 3).

Cholnoky (1958a) developed a method by which a change in the amount of pollution was indicated by

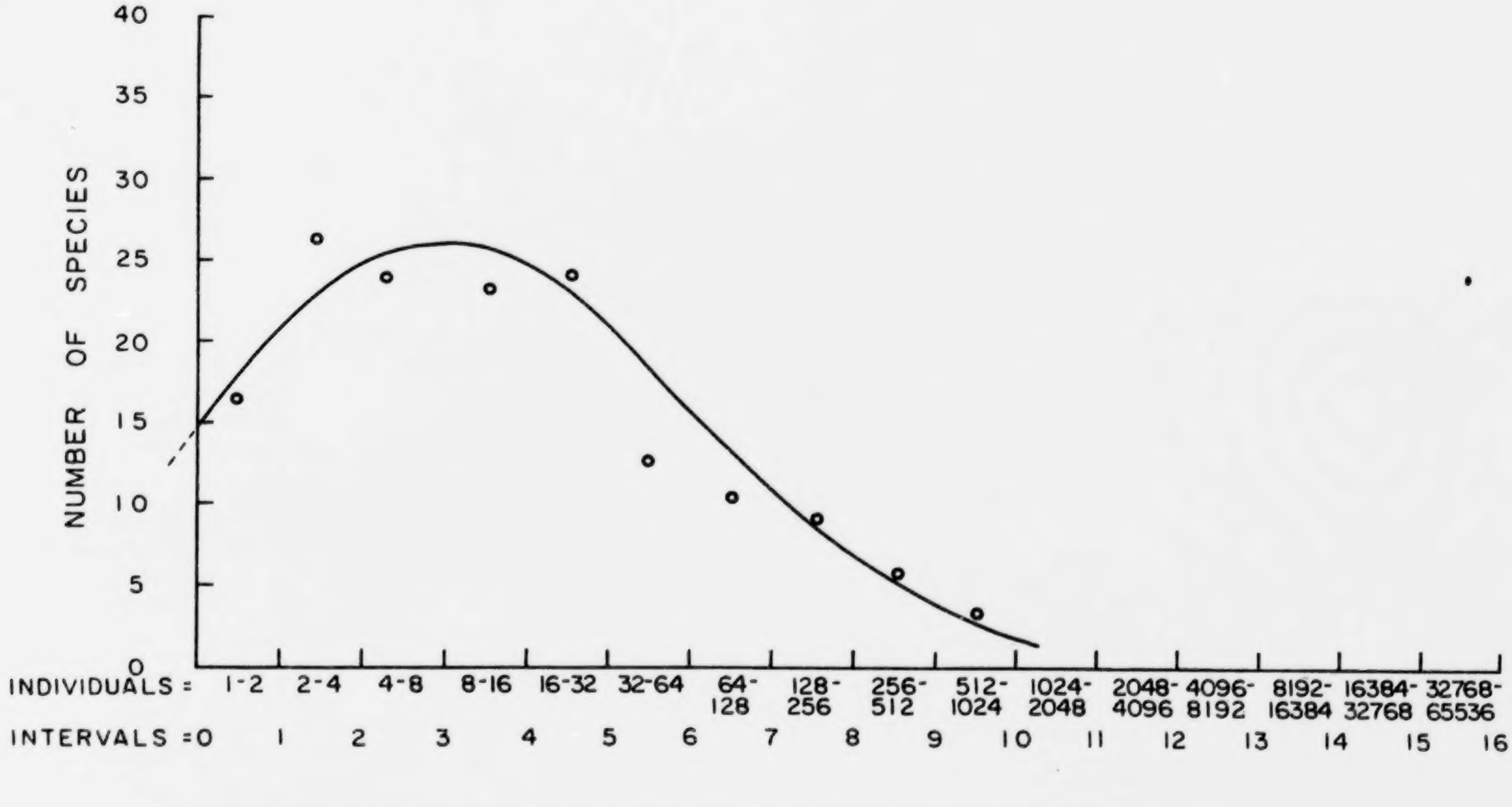

Figure 1. The structure of a natural diatom community, Ridley Creek, Pennsylvania.

Table 1. SUMMARY OF CATHERWOOD DIATOMETER READINGS AT STATION 1 – SAVANNAH RIVER (OCTOBER 1953 TO JANUARY 1958)

Date	Specimen number in modal interval	Species in mode	Observed species	Species in theoretical universe
Oct. 1953	4 to 8	22	150	178
Jan. 1954	4 to 8	19	151	181
Apr. 1954	2 to 4	24	169	200
July 1954	2 to 4	23	153	193
Oct. 1954	4 to 8	21	142	168
Jan. 1955	4 to 8	19	132	166
Apr. 1955	2 to 4	25	165	221
July 1955	2 to 4	20	132	180
Oct. 1955	2 to 4	27	171	253
Jan. 1956	2 to 4	30	185	229
Apr. 1956	4 to 8	35	215	252
July 1956	2 to 4	24	147	185
Oct. 1956	2 to 4	23	149	206
Jan. 1957	2 to 4	29	177	233
Apr. 1957	2 to 4	21	132	185
July 1957	4 to 8	29	181	203
Oct. 1957	2 to 4	25	157	232
Jan. 1958	2 to 4	27	152	212
(Apr. 1954 to 1958 averages)	—	24	151	194

changes in the percent of dominance of certain species, such as species of the genus *Nitzschia* (Figure 4). Cholnoky (personal communication) stated that the general ecological conditions in the stream he studied did not vary much throughout the year. Patrick (in press) stated this may be possible if the environmental characteristics remain relatively constant or if one knows all other environmental factors influencing diatom growth, but that one usually does not have this much information; furthermore,

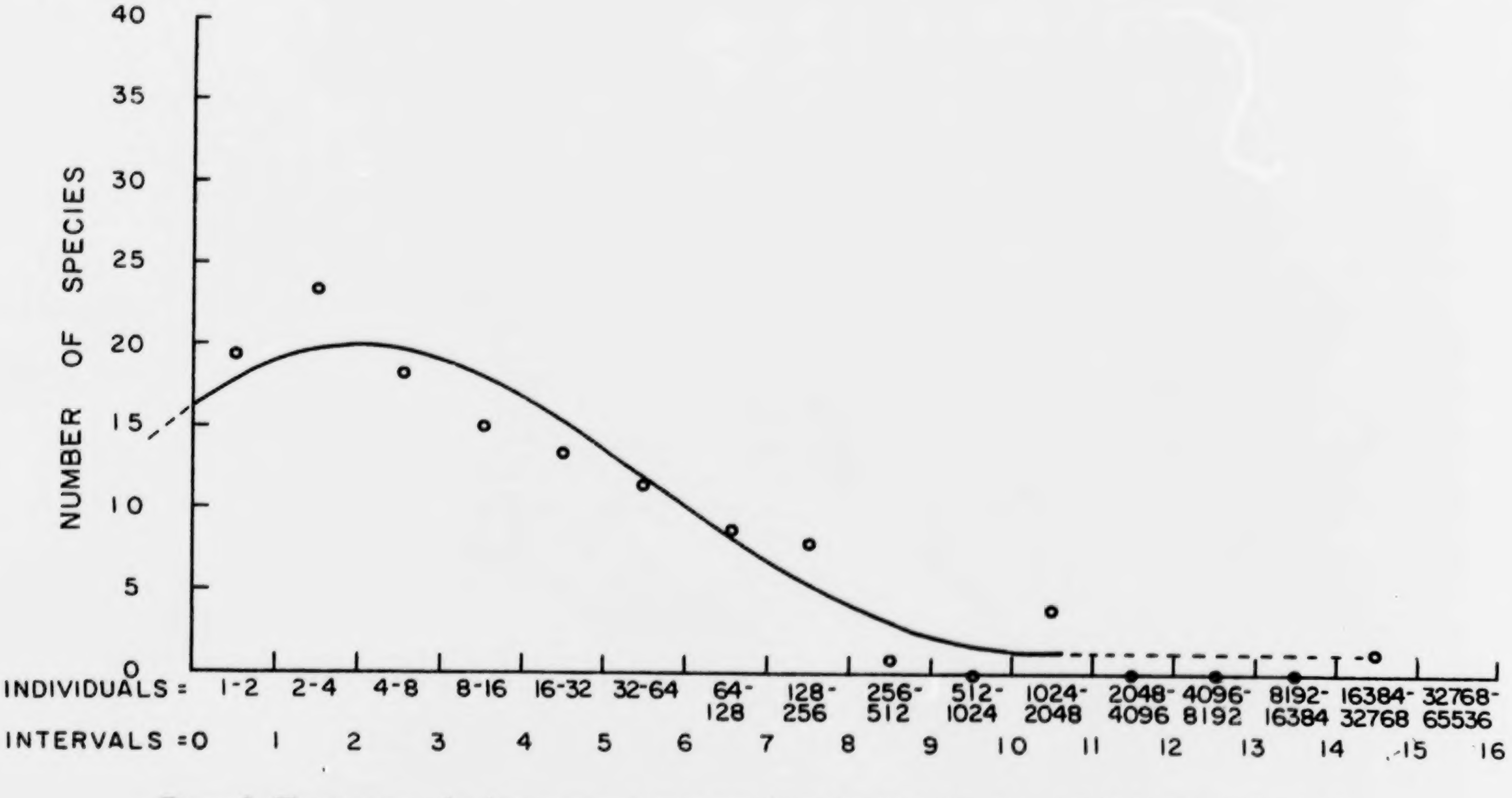

Figure 2. The structure of a diatom community in a moderately polluted environment, Nobs Creek, Maryland.

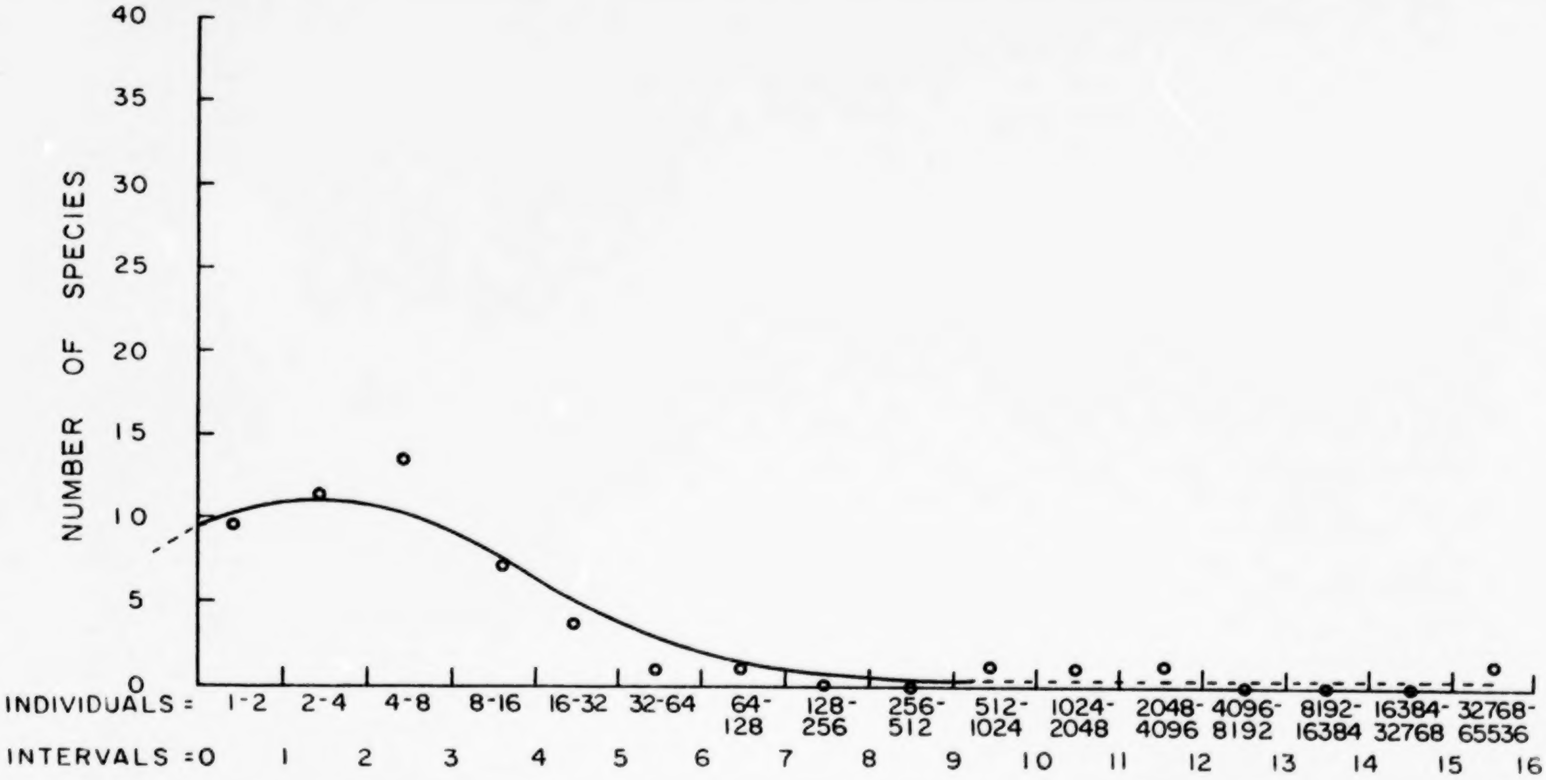

Figure 3. The structure of a diatom community in a severely polluted environment, Back River, Maryland.

there may be considerable change in the dominant species owing to factors of environment other than changes in the pollution load. However, the total percent of the population composed of dominant species, rather than the population size of any given species, is a valuable criterion for judging the effect of pollution.

The use of changes in sizes of populations of one or a few species might be more possible if one were dealing with a group such as fresh-water bryozoa or oligochaete worms in which the numbers of species that might live in a given area are smaller and thus the species change would not be as great with small changes of the enviornment, because species competition is much less. This does not alter the fact, however, that natural changes in the environment may bring about the exclusion of a species.

Hentschel (1925), in pointing out the difficulties in using indicator species of animals, emphasized the fact that characteristics of the natural environment may be very similar to those in a moderately polluted environment. He also pointed out that conditions in the natural environment may limit the development of a species in similar ways as pollution, and as a result, changes in abundance of a species may not be related to changes in pollution. He stated that, although certain strains of a species may be resistant or sensitive to pollution, other strains may behave in quite a different manner. Butcher (1947), Hustedt (1957), and others have pointed out that very few, if any, diatoms are characteristic of a given kind of pollution, but rather that some diatoms are tolerant or resistant to many kinds of pollution and thus occur in polluted zones where most species are absent.

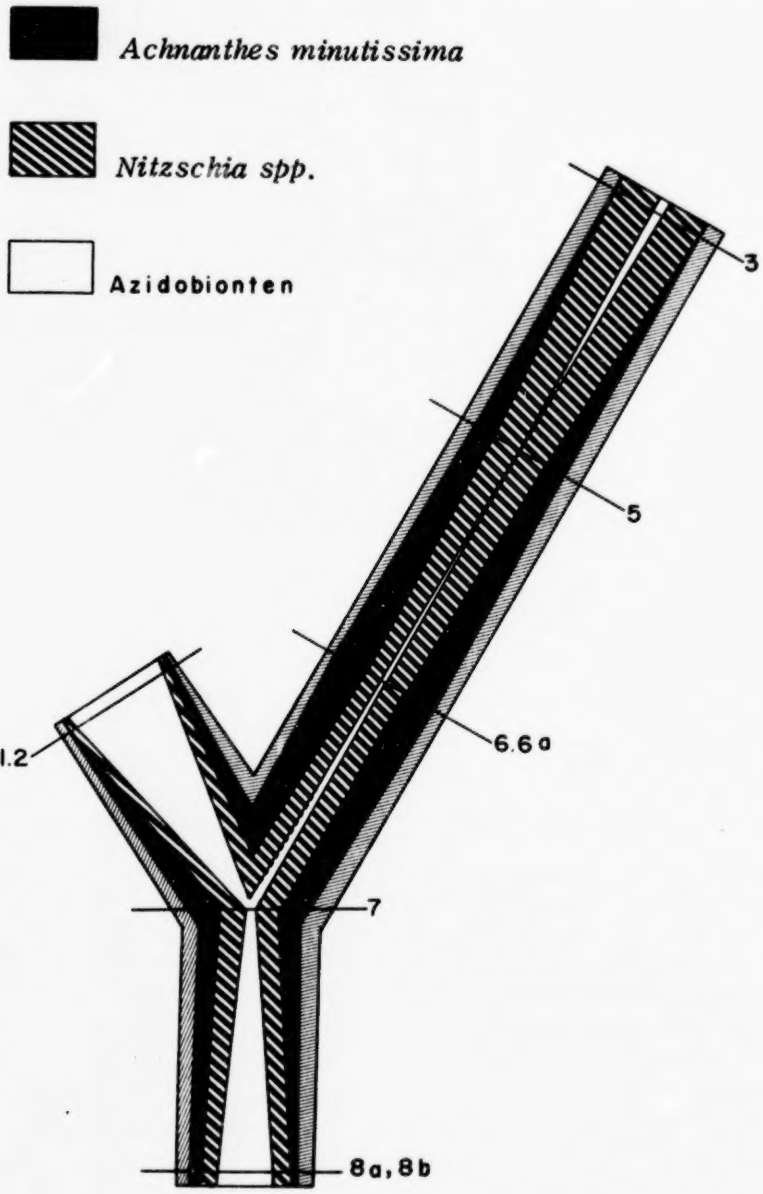

Figure 4. Changes in the dominance of certain diatoms because of pollution (after Cholnoky, 1958a).

Cholnoky (1960) stated that one must know a great deal about the general physiology of the diatom before he can state that it is characteristic of a given chemical condition in water. He came to the conclusion that Hornung (1959) stated: That the total conductivity of the medium in which a diatom lives is often more important than a given ion such as chloride in determining the presence of a given species.

The kinds of algae species that are indicators of pollution have been designated by many workers. Species characteristic of four major stages or conditions encountered in the assimilation of organic wastes have been recognized. These have been discussed by workers such as Kolkwitz and Marsson (1908), Schroeder (1939), Kolkwitz (1950), Liebmann (1951), Hustedt (1957), Hornung (1959), Fjerdingstad (1950, 1960), and Cholnoky (1958a,b).

Other workers have reported various algae that they have found to be characteristic of various degrees of pollution caused by industrial wastes or a mixture of industrial and sanitary wastes. Butcher (1955) reported *Chlorococcum* sp. grows well in the presence of fairly high copper concentrations. Schroeder (1939) reported *Stigeoclonium tenue* as being sensitive to 0.8 milligram of copper per liter of water, whereas *Fragilaria virescens, Synedra ulna, Achnanthes affinis, Neidium bisulcatum, Navicula viridula, Cymbella naviculiformis, C. ventricosa, Gomphonema parvulum,* and *Nitzschia palea* were found to live in 1.5 milligrams of copper per liter. Two milligrams of copper per liter was tolerated by *Achnanthes affinis, Cymbella ventricosa,* and *Nitzschia palea.* Corbella et al. (1958) found that *Achnanthes nodosa* was very tolerant to copper and ammonia. Jones (1958) found that in lead concentrations of 0.4 to 0.5 mg/l the only common algae were *Batrachospermum* and *Lemanea mammellosa.*

Schroeder (1939) found the following diatoms at pH 3.6 to 4, and with an Fe_2O_3 concentration of 5.7 mg/l: *Eunotia exigua, E. trinacria,* and *Navicula subtilissima.* In flowing water with a fairly high $Fe(OH_3)$ content were *Mougeotia, Ulothrix tenera, Gallionella ferruginea, Leptothrix ochracea, Eunotia exigua, E. lunaris, E. trinacria, Pinnularia subcapitata var. hilseana, and Surirella linearis.*

Schroeder (1939) also found that many green algae are more sensitive than diatoms are to H_2S. *Stigeoclonium tenue* could not tolerate a concentration of 0.2 mg/l, whereas *Achnanthes affinis, Cymbella ventricosa, Hantzschia amphioxys,* and *Nitzschia palea* could tolerate H_2S concentrations of 3.9 mg/l; *Cyclotella meneghiniana, Neidium bisulcatum, Navicula minima, Nitzschia ignorata, N. tryblionella var. debilis* and *Surirella ovata* var. *sabina* could tolerate H_2S concentrations of 1.5 to 3.7 mg/l. Blum (1957) reported the following algae as resistant to chromium and other pollutants: *Stigeoclonium tenue, Tetraspora* sp., *Closterium aceorsum, Nitzschia palea, Nitzschia linearis, Navicula atomus, Navicula cuspidata, Euglenia sociabilis, E. viridis, E. acus, E. oxzuris, E. stellata.* Patrick (report on **USPHS** grant, 1960) found 0.2 to 0.4 mg of chromium per liter toxic to *Novicula seminulum var. hustedtii.*

In a series of experiments carried out in the Limnology Department of the Academy of Natural Sciences (USPHS grant), *Navicula seminulum* var. *hustedtii* was found to have the following sensitivities to various chemicals. The concentrations that caused 50 percent reduction in the amount of division occurring in 5 days in laboratory tests are as follows: Cyanide, 0.28 to 0.49 mg/l; soluble zinc, 1.3 to 4.05 mg/l; phenol, 251 to 261 mg/l; and naphthenic acid, 28 to 79 mg/l. Variation in the concentrations of a given chemical was due to different hardness of water and different temperatures under which the tests were conducted.

Kolbe (1932) stated that *Achnanthes minutissima* and *Gomphonema olivacuum* often develop large populations that are associated with large amounts of calcium carbonate. Cholnoky (1958a) considered *Achnanthes minutissima* as indicative of a considerable amount of oxygen in the water. Palmer (1960) gave a list of genera of bluegreens that are characteristic of water high in nitrogen and low in calcium.

In summarizing the results of research in this field, it is apparent that certain species of blue-green algae, red algae, green algae, and diatoms may be useful in qualitatively indicating certain chemical and physical conditions of the water as they relate to pollution and that most of these species are tolerant to various types of pollution rather than indigenous to them. The most reliable method in considering algae as indicators of pollution is to study the algal community as a whole and consider the kinds of species, relative sizes of the populations of the various species, and numbers of species. Furthermore, if one wants to compare the algal flora in various areas it is necessary to base conclusions on similar segments of the algae community.

When using names to describe a condition characterized by many kinds of data one arbitrarily limits many continual gradients of conditions. Thus it is impossible to describe briefly a condition by any name such as "α-mesosprobic," and have it be infallible. For this reason, in 1949 I adopted such terms as "healthy" and "semi-healthy," "polluted," and "very polluted" to connote the variability one might expect in the organisms and conditions present, and yet indicate a degree of degradation that had taken place.

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DISCUSSION

A question arose whether the definition of the word "pollution" should be based on the present economic or practical use of the water. It was noted, however, that we ordinarily assume a large degree of the water usage will be in the future, and, therefore, care must be exercised to account for the condition of the bodies of all water.

In considering the advantages of a biological index of pollution over a chemical one, it was brought out that chemicals ordinarily have a more rapid response to changes than the algae. Although the chemist is able to tell what specific chemicals are present at a given time, he might often miss things. The biological method will at least indicate that something has happened and give you an overall picture. In reality, the two indices complement each other.

It was noted that classifying pollution according to zones or organisms is fashionable in Europe, but it was suggested that perhaps the workers in the United States are not completely familiar with this type of classification. Although pollution may occur in many types and kinds of streams and some people are ever trying to apply the indicator organism concept to lakes, one cannot really compare a mountain stream with a river such as the Ohio. Therefore, it is impossible to use any particular organism or group of organisms as pollution indicators for all of the many types of aquatic communities. Also, there appears to be a great deal of disagreement among authors about the use of teminology in pollution studies.

The number of kinds of algae considered to be pollution tolerant is generally quite limited for any one area or survey but becomes large when all of the results of many investigators are combined. When a compilation was made of the reports of 110 workers, a total of more than 50 genera were listed as being tolerant to sewage or related conditions. The genera *Euglena* and *Oscillatoria*, however, were listed as the most tolerant by over 50 percent of the workers. In closing this area of discussion, it was suggested that we are working too hard with complex systems and calculations, and, in reality, a good biologist can merely look at a stream and get a good idea of its condition.

It was pointed out that there is a fundamental difference in a community having a few species and one having many.

A question arose whether there is a quantitative relationship between a biological index and the organic material present in a stream. An example was given of a river having a relatively low pH and also being polluted. An examination of the species present can readily tell you whether you have pollution or not, by whether there are acid-loving species present or pollution-tolerant species present.

Again the question was raised whether anything but the dominant group is significant (in a microphyte community). In answer to this it was stated that all species, whether dominant or not, can tell you whether a condition is toxic or not.

In controlling algae in reservoirs and ponds, the engineers try to control nutrients affecting the growth of algae and at present are trying to control the phosphate content. The question was asked if phosphate control is effective in controlling algal growth. It was pointed out that there are many nutrients involved and it would be of little value to control the phosphate without controlling the nitrogen. It was also mentioned that the algae that are considered to be the worst odor producers do not require much phosphate.

SOME REMARKS ON A NEW SAPROBIC SYSTEM

*E. Fjerdingstad**

At the Second Seminar on "Biological Problems in Water Pollution" held in Cincinnati in 1959, Professor Erman A. Pearson gave a very interesting survey of the antagonism between the estimation of pollution by engineers and by biologists. In his search for a quantitative method for biological assays of the waters, he went on to say that "there should be a minimum core of biological measurements that would suit every situation just as there is with respect to physical and chemical assays."

Professor Pearson also proposed a working scheme for estimation of pollution containing the following biological items: (1) Coliform; (2) phytoplankton; (3) zooplankton; (4) fish; (5) Biomass; and (6) identification and enumeration of genus and species of organisms, whose numbers are equal to or more than 10 percent of total population.

From the point of view of biologists, these demands are very great and time consuming and would necessitate the participation of several specialists. Besides, exact determination of the biocoenosis of sessile microorganisms would hardly be possible at present in practical work. Therefore, the method could only be used to determine the planktonic population numerically.

THE MATHEMATICAL METHOD OF REPORTING BIOTIC DATA

In some papers published in recent years, authors converted the biological estimation of pollution into numerical values (Pantle and Buck, 1955; Beck, 1955; Knöpp, 1954). They were forgetting that biological communities are complex systems and cannot be reduced to exact numerical values to be entered into neat columns alongside the analytical results.

It is a common feature of these systems that the individual species is given a qualitative numerical value depending on the saprobic zone to which the species belongs and a numerical value representing the estimated quantities in which it occurs.

Pantle and Buck thus use the following numerical values for the qualitative evaluation of the saprobic zone ("s") -- 1 = oligosaprobic; 2 = β-mesosaprobic; 3 = α-mesosaprobic; and 4 = polysaprobic. The estimated quantitative evaluation "h" is denoted as follows -- 1 = occurring incidentally; 3 = occurring frequently; and 5 = occurring abundantly. For this purpose a mean saprobical index "S" is calculated for each locality.

$$S = \frac{\Sigma sh}{\Sigma h}$$

The authors give the following numerical values for the individual zones: 1.0 to 1.5 denotes the oligosaprobic zone; 1.5 to 2.5 denotes the β-mesosaprobic zone; 2.5 to 3.5 denotes the α-mesosaprobic zone; and 3.5 to 4 denotes the polysaprobic zone.

Mathematical manipulations that are based on the numerical values ascribed to the individual species are far from exact because the different ecological valencies of the species actually argue against the application of an exact mathematical interpretation. Even if the other objections were disregarded it is obvious that these values cannot tell anything about the polysaprobic zone, which is particularly characterized by the great differences between maximum and minimum load.

Some authors (Caspers and Schulz, 1960; Bringmann and Kühn, 1960) repudiate the mathematical methods as does the present author.

DEPENDENCE OF THE MICROCOMMUNITIES ON BIOCHEMICAL FACTORS

Many of the organisms occurring in the polluted waters are able to utilize a great variety of nutrients. For others the choice is limited since they are dependent upon the presence of specific substances.

The interactions among different species (due to extracellular substances) occurring at a locality is probably of great importance in the composition of the local community of organisms.

Vitamins also seem to be essential to the growth of algae; e.g., *Euglena gracilis* requires thiamine and, in addition, vitamin B_{12} to produce optimum growth.

Hutner, et al. (1956) state that it may prove to be as important to account for the contents of certain vitamins and other organic compounds as to measure temperature, concentrations of nutrient salts, and other "classic variables."

As the algal population changes with the decreasing pollution of a receiving water, it must be said that by considering solely the inorganic salts, as has hitherto been done, it is impossible to appreciate the causal principle.

The requirement that the biological estimation of pollution should be correlated with chemical factors cannot be fulfilled at present, for routine chemical analyses can only illustrate an approximate relationship.

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A NEW SAPROBIC SYSTEM

Kolkwitz and Marsson's saprobic system (1908) gave a list of indicator species for biological estimation of pollution. Indicator species are not particularly suitable for determining the zones of pollution, however, because the adaptation possibilities of a species have, as a rule, very wide ecological limits. For this reason a species will often occur in several saprobic zones.

It is clear, however, that the optimal conditions for the presence of a species are generally very closely delimited in contrast to the ecological limits of its possible occurrence. This suggested that the community-forming organisms might form the basis of the estimation since community formation must be analogous to the optimal living conditions of the species. In this case, however, it is essential to distinguish sharply between the autecological (the single species) and the synecological (community) aspects. For only by doing so will it be possible to obtain a further development of the saprobic system.

As to the autecological designation of the relationship of an organism to pollution, the author has chosen these designations:

saprobiontic:	species that occur (in large numbers) only in heavily polluted waters.
saprophilous:	organisms that occur generally in polluted waters but may occur also in other communities; i.e., organisms that to a certain extent are indifferent.
saproxenous:	organisms that occur generally in biotopes other than polluted ones but may survive even in the presence of pollution.
saprophobous:	organisms that will not survive in polluted waters.

In this system the relationship of each particular species to pollution will allow for a better understanding than the traditional division corresponding to the appurtenant saprobic zones will.

As to the pollutional zones, we have hitherto had too few, a situation felt to be of importance especially in the polysaprobic zone where there are such considerable and decisive differences between, for instance, the last and the first part of the zone. Finally, Kolkwitz and Marsson's system contains no zone for the undiluted faecal water in which oxidation and mineralization have not begun.

The strictly delimited zone for undiluted faecal water is named the coprozoic zone. Moreover, a distinction is made between an α-polysaprobic, a β-polysaprobic, and a γ-polysaprobic zone; an α-mesosaprobic, a β-mesosaprobic, and a katharobic zone; there are altogether nine zones.

By extending the zone number to nine, increased possibilities are achieved with regard to indicating the amount of pollution. Consequently, for the nine zones there is a corresponding series of microorganism communities (Table 1).

Table 1. SURVEY OF THE SAPROBIC ZONES AND THE CORRESPONDING COMMUNITIES

Zone I. Coprozoic zone

- a: the bacterium community
- b: the *Bodo* community
- c: both communities

Zone II. α-polysaprobic zone

- 1: *Euglena* community
- 2: Rhodo-Thio bacterium community
- 3: pure Chlorobacterium community

Zone III. β-polysaprobic zone

- 1: *Beggiatoa* community
- 2: *Thiothrix nivea* community
- 3: *Euglena* community

Zone IV. γ-polysaprobic zone

- 1: *Oscillatoria chlorina* community
- 2: *Sphaerotilus natans* communities

Zone V. α-mesosaprobic zone

- a: *Ulothrix zonata* community
- b: *Oscillatoria benthonicum* community *(Oscillatoria brevis, O. limosa, O. splendida* with *O. subtilissima, O. princeps*, and *O. tenuis* present as associate species)
- c: *Stigeoclonium tenue* community

Zone VI. β-mesosaprobic zone

- a: *Cladophora fracta* community
- b: *Phormidium* community

Zone VII. γ- mesosaprobic zone

- a: Rhodophyce community *(Batrachospermum moniliforme* or *Lemanea fluviatilis)*
- b: *Chlorophyce community (Cladophora glomerata* or *Ulothrix zonata* (clean-water type)

Zone VIII. Oligosaprobic zone

- a: Chlorophyce community *(Draparnaldia glomerata)*
- b: pure *Meridion circulare* community
- c: Rhodophyce community *(Lemanea annulata, Batrachospermum vagum* or *Hildenbrandia rivularis)*
- d: *Vaucheria sessilis* community
- e: *Phormidium inundatum* community

Zone IX. Katharobic zone

- a: Chlorophyce community *(Chlorotylium cataractum* and *Draparnaldia plumosa)*
- b: Rhodophyce community *(Hildenbrandia rivularis)*
- c: lime-incrusting algal communities *(Chamaesiphon polonius* and various *Calothrix* species)

Legend: a, b, c -- as alternatives
1, 2, 3 -- as differences in degree

CHEMICAL REASONS FOR THE ZONE DIVISION

With some reservations because of the abovementioned shortcomings of the chemical analyses of sewage to characterize the zones of a saprobic system, there may be said to exist, however, a certain chemical basis for the saprobic system developed by the author. By neglecting extreme analytical values that may be considered the result of some failure or other, e.g., metallic poisons, very large precipitations, or sudden thawing of snow that greatly dilutes the sewage, a better agreement may be obtained between the chemical analyses and the biological division into the author's nine zones.

On the basis of a considerable number of analyses, Figures 1 through 4 represent graphically the average course of some chemical actions in the zones as well as maximum and minimum values.

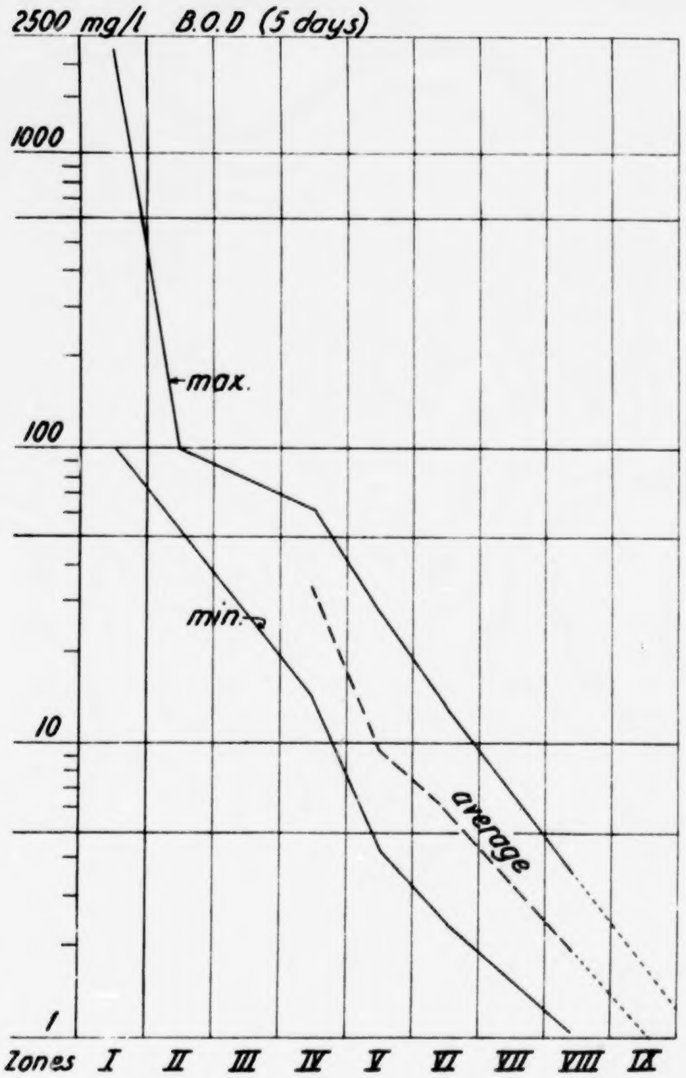

Figure 1. Diagram representing maximum, minimum, and mean values of BOD determinations.

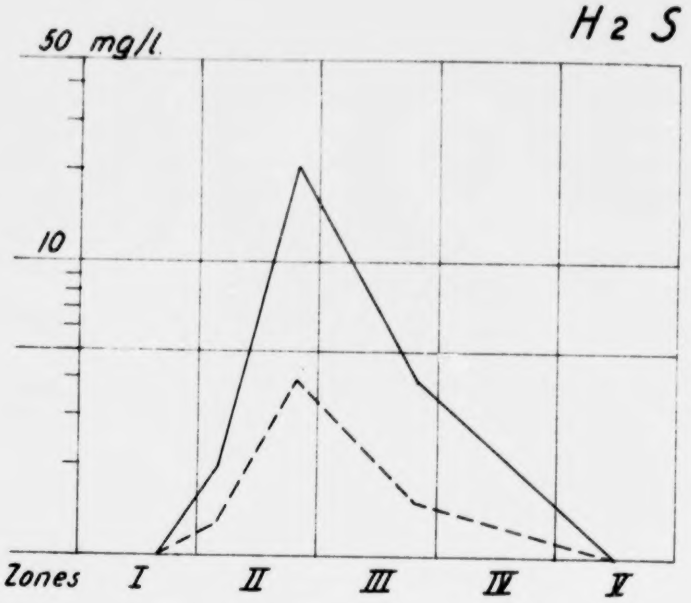

Figure 2. Diagram representing maximum and minimum values of hydrogen sulphide content in Zones I to V.

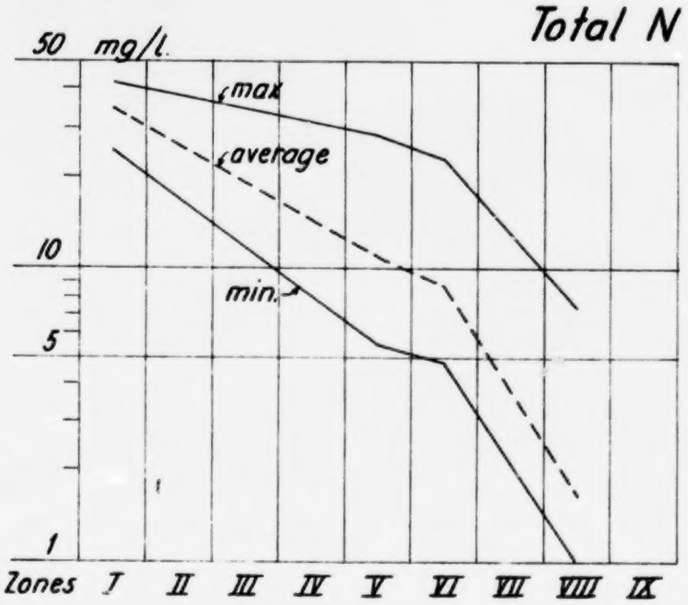

Figure 3. Diagram representing maximum, minimum, and mean values of total nitrogen content.

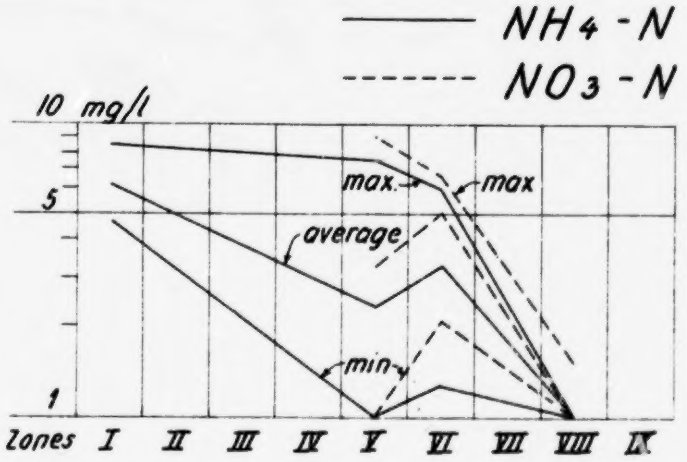

Figure 4. Diagram representing maximum, minimum, and mean values of ammonia nitrogen and nitrate nitrogen.

POLLUTION BY INORGANIC MATTER

Each type of industrial waste containing inorganic substances has its special "appearance" and its communities, depending on the type and extent of the compounds in the effluent (especially potentially toxic substances). All that can be done at present with this type of industrial waste is to distinguish between Zone I, the chemotoxic zone, and Zone II, the chemobiontic zone. In the former, no organisms occur because of the amount of toxic substances; in the latter, some organisms are found.

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THE SIGNIFICANCE OF MACROINVERTEBRATES IN THE STUDY OF MILD RIVER POLLUTION

*H.B.N. Hynes**

I have the impression, after reading the transactions of the first two seminars in this series, that perhaps the last word on biological indicators of pollution has been written. It also seems that either nobody quite knows just what is meant by pollution or everybody means something different. Doudoroff and Warren (1957), in particular, stressed that if, by pollution, is meant damage to fisheries, it is only rational to study fishes; if deterioration in the usefulness of water for some other purpose is meant, one should study the water from that particular viewpoint. This is sound sense, but it can, I submit, be carried too far. One could, for instance, ask the chemists why they measure BOD. Their object in doing so is, fundamentally, to determine the effect of the effluent on oxygen concentration in the receiving water, so why not measure only that? I think a chemist might reply that it was easier and more informative to work with BOD, since it is less liable to be disturbed by extrinsic factors. I feel that the biologist can also say that, at least initially, small organisms are easier and more satisfactory to work with than fish since they are relatively simple to sample and they do not move around very much. If they show signs of alteration. it may be worth going on to see if the alteration also applies to other properties of the water, such as its ability to support fish or its usefulness for water supply. If no great alteration is found in the invertebrates, it seems reasonable to assume that none has in fact occurred to other characteristics of the river. I can imagine no type of pollution that would make water unsuitable for industrial, domestic, agricultural, or fishery purposes without having some detectable effect on the flora and fauna. Perhaps radioactivity could do this, although I doubt it; anyway, it would be a strange organization that sent out only a biologist to look for radioactive pollution if it were suspected.

I agree that pollution is not a word with any absolute meaning and that each case must be looked at by itself, taking all sorts of factors into account; I do not subscribe to the school of thought, to which Doudoroff and Warren relegate Dr. Patrick, that considers any alteration to the biota as pollution, although that is in fact a reasonable interpretation of the common law in both Great Britain and the United States. I do believe, however, that study of fauna and flora is a very useful and rapid tool in the investigation of pollution. It can also be a very delicate one, but like all such instruments it must be used with care, intelligence, and skill.

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Liebmann (1951, 1961) is the great contemporary exponent of the idea first advanced by Kolkwitz and Marsson (see Kolkwitz, 1950, for a recent exposition) that microorganisms can be used to indicate organic pollution. This is certainly true, although I would not agree with the details of their zonation, which seem altogether too theoretical, and which have often been shown not to apply to particular cases. Despite difficulties of technique and identification that arise in the use of microorganisms (Lackey, 1957), and the fact that most of them must be studied alive, they do offer a means of detecting, and to some extent of quantifying, fairly heavy concentrations of organic matter, although they are not as useful for other types of pollution. Patrick (1957) advocates the use of diatoms, which do not suffer from some of the disadvantages of other small organisms. These small plants may prove to be extremely useful, although I would suggest that it is dangerous to confine a study to just one group of organisms when dealing with the whole range of possible pollutants. To cite two examples from my own field of work, it is well known that stoneflies are eliminated by very low dosages of organic matter and so are often taken to indicate well-oxygenated water suitable for fish; but they are unaffected by concentrations of heavy metals that are quite lethal to fish (Jones, 1940 a and b.). Similarly, the sludge worms *Tubifex* and *Limnodrilus* thrive in organically polluted water but are killed by small amounts of heavy metals.

Gaufin (1957) has given a general account of the occurrence of aquatic insects in relation to organic enrichment; the main principles of the use of these and other invertebrates as indicators of organic pollution are now well understood. It is clear, however, that further work on basic biological problems will enhance our understanding. Thus, for instance, my colleague Dr. R.O. Brinkhurst, in a study of the rather neglected tubificid worms, found that the specific composition and the proportion of the species present change as one passes from severe to less severe organic pollution. I believe that further knowledge of the Chironomidae also might make the larger invertebrates more useful as indicators in heavily polluted water. Particularly in this zone of heavy organic pollution is so much similarity found among the fauna in widely separated parts of the world. Thus, *Tubifex*, *Limnodrilus*, and *Chironomus* species are reported in polluted areas as far apart as Java (Vaas and Sachlan, 1955), South Africa (Allanson, 1961), Europe (Hynes, 1960), and North America (Gaufin and Tarzwell, 1956).

Nevertheless, even under conditions of fairly heavy enrichment, there can be no rigid rules, because regions differ in their fauna, and rivers and effluents differ from one another. To ask, as did Pearson (1960), that biologists should produce quantitative data that are universally applicable is like crying for the moon. He seems to have been unfortunate in his encounters with biologists in that some of them appear to have been very narrow-minded, some very readily overwhelmed by difficulties, and others overenthusiastic and unaware that vast numbers of measurements lead only to indigestible piles of data. Here I agree with Doudoroff and Warren that, if much time and effort is to be spent in investigations, one should measure only what one wants to know.

The macroinvertebrate survey, which is relatively quick and easy to make, tells one if it is worth investigating further. Since it is based on no hard and fast system, it is flexible and allows the investigator to look afresh at each problem and interpret the data in the light of prevailing circumstances. I would go so far as to say that this sort of interpretation is also necessary for strictly numerical chemical and physical data because rivers vary in their susceptibility to pollution. A load of organic matter that would be damaging in a trout stream may be quite acceptable in a sluggish base-level river simply because the inhabitants of rapid, cool streams are very sensitive to diminution of dissolved oxygen and to silting, whereas those of sluggish warm streams are much less so. Similarly, salmonid fishes seem to be more sensitive to a wide range of poisons than are fishes of other families. Thus, I maintain that even the numerical data of the chemists are not the strictly quantitative parameters of pollution that they seem to be at first sight.

The larger invertebrates have, in a sense, "escaped" from inclusion in the Kolkwitz and Marsson Sabrobiensystem, largely because very few were mentioned in the original papers, and many of those that were included were misidentified -- a fault that Liebmann has done little to correct. So, although some European workers (e.g., Huet, 1949) have produced lists of animals said to be characteristic of certain levels of pollution, macroinvertebrates have not become deeply involved in the doctrine. Many of them, however, show clear reactions to organic pollution. It is also well established (Carpenter, 1924; Pentelow and Butcher, 1938) that they react to poisons and simple salts (Liebmann, 1951; Lafleur, 1954), so there is a reasonable chance that they can serve to detect all kinds of pollution. Simple surveys of the invertebrates have, as I hope to show, great sensitivity, especially if they are quantitative; I know that they give useful results in Britain (Hynes, 1961b; Hynes and Roberts, 1962) and in Africa (Hynes and Williams, 1962). I believe, therefore, they can be useful anywhere, but the interpretation of results does require biological knowledge. In a subject as complex as ecology, I do not see how that can ever be avoided.

It is well known that unaltered "natural" rivers have a definite type of fauna. Details vary from one region to another, but in general the same sort of life forms recur even though, as Illies (1961) has recently shown, similar forms may have been evolved by widely differing organisms in different continents. It is also well established that, as one goes down a river from source to base level, one passes through a sequence of more or less well-defined zones, each with its characteristic but broadly similar fauna (Illies, 1955; Schmitz, 1957; Hynes, 1961a). It is clear, therefore, that slight changes in water hardness, silt deposition, oxygen regime, and so on, induce slight faunistic changes. These changes affect different creatures at different levels, and initially involve only alterations in relative abundance. In Europe and doubtlessly in North America, for instance, there is a decline in the importance of Plecoptera and an increase in the importance of Ephemeroptera as one goes downstream.

We can use the method that served us in discovering this sort of thing to detect the obverse changes in rivers that are altered by man. This can be done simply and quickly, and is a very valuable first source of information since it shows if a particular effluent is in fact causing an alteration, how far that alteration extends, and whether it is becoming more or less marked. It does not and cannot indicate whether fish are affected or whether water is altered for industrial use any more than can routine chemical analysis of a mildly polluting effluent, but if it reveals little change in the invertebrate fauna, it is reasonable to assume that the effects are not adverse. Similarly, if the change leaves a fairly normal river fauna, albeit different from the one that was there before, it is reasonable to assume that some types of fish, at least, will thrive and that the water is still usable, although perhaps not for the same purposes as before. Here once more we come hard up against the question of what we mean by pollution. The degree of alteration that can be permitted is a matter of administration at a high level.

If one collects a series of samples of invertebrates at points spaced along a river, changes in fauna are readily observed. Collections must be quantitative and from the same sort of substratum at each point, but how they are made can vary according to circumstances. Ideally, areal samples with a sampler like that of Surber can be used, and they are worthwhile if great accuracy is needed as well as an estimation of confidence limits. Usually, a simple collection with a hand net plied in a fixed manner for a fixed time suffices. If the water is too deep for wading, the technical problems are greater, but various grabs and dredges can be used. All types of samplers are selective to some extent (Macan, 1958; Hynes, 1961a), but the collections should be comparable if the same methods are used at all stations; they only cease to be so if there are marked changes in rate of flow below the outfall.

Figure 1 graphically shows the results of four surveys of a Welsh river when it was recovering from pollution by a chemical works whose effluent

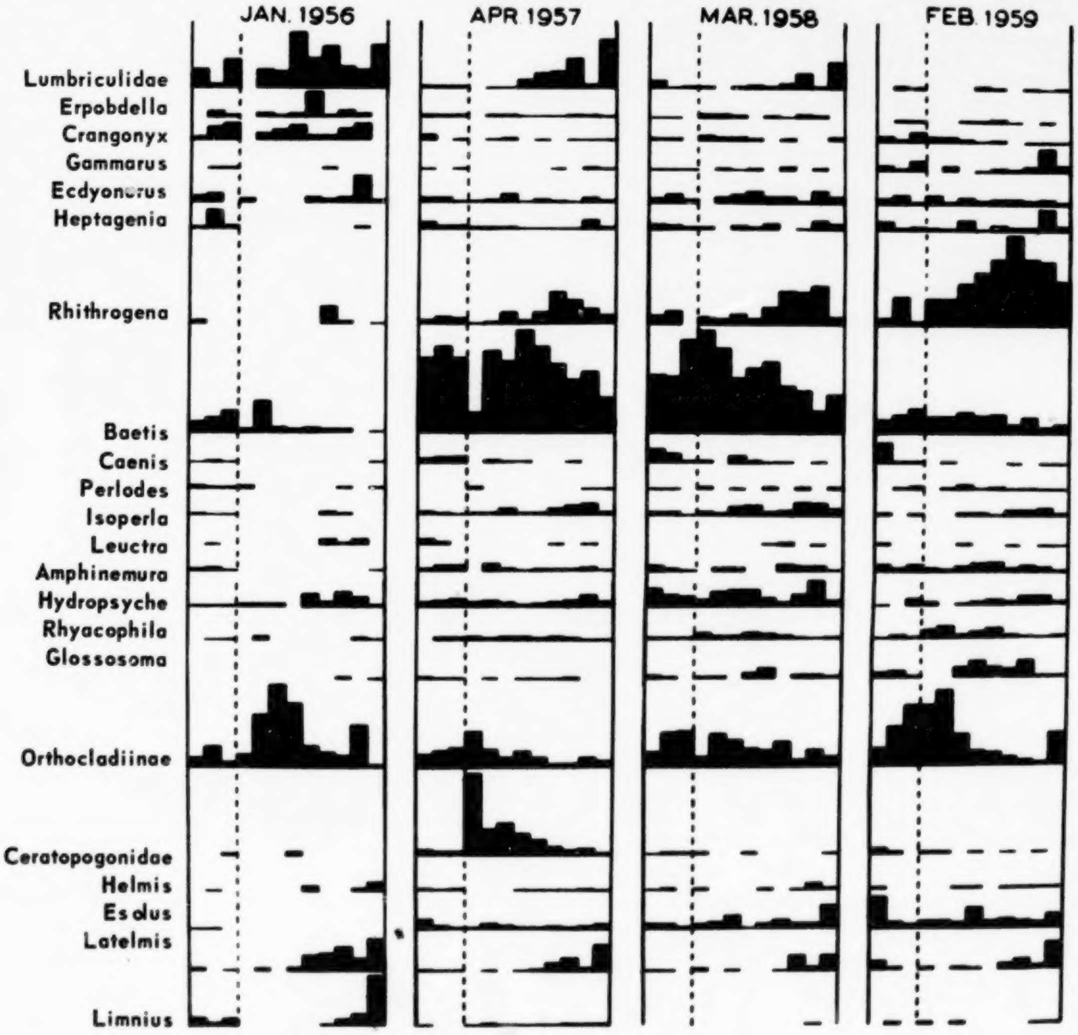

Figure 1. The percentage composition of the principal members of the fauna of a British river in 4 successive years. The river flows from left to right in each set of histograms. The 12 stations, shown juxtaposed, were 1.3, 0.4, and 0.2 miles above the outfall from a chemical works, shown by dotted lines, and 0.2, 0.6, 1.1, 2.6, 4.7, 5.9, 7.8, 10.2, and 16.0 miles below it. At each station, the vertical height of each histogram is proportional to the percentage of the total number of specimens caught at that station. The figure is based on the following total numbers: 1,987, 1956; 10,805, 1957; 3,487, 1958; and 3,836, 1959.

contained various organic compounds, mostly phenolic. The factory had installed a modern and effective treatment plant, which was still being improved during 1955, 1956, and early 1957. The histograms show the percentages of the total fauna collected at each station; only the more common animals are shown. The stations were spaced at increasing distances from the outfall, three above (1.3, 0.4, and 0.2 miles) and nine below (0.2, 0.6, 1.1, 2.6, 4.7, 5.9, 7.8, 10.2, 16.0 miles); a standard 10- or 15-minute collection was made with a hand net at each station. The time spent varied with the river level; in 1956 and 1959, the tests took 15 minutes because the level was high, and in 1957 and 1958 they took 10 minutes because the level was relatively low. The collections at 0.2 mile below the outfall were made on the left side of the river, in the path of the effluent, since the effluent did not become fully mixed until some distance downstream.

In 1956, the effluent was having a fairly marked effect on a number of animals: many were absent from the first station below the outfall; even some that were present (e.g., *Ecdyonurus* and *Perlodes)* were almost certainly strays, perhaps from the opposite bank, since they were absent from several stations downstream. Most notably, the Ecdyonuridae (Heptageniidae), Plecoptera, and Helmidae were absent for some distance, but *Baetis*, mostly the species *rhodani*, was reduced in importance only below the outfall. In North America a species of *Callibaetis* has been recorded as occurring in organically polluted water (Gaufin and Tarzwell, 1956) and (that) *Baetis harrisoni* has been reported from midly polluted water in South Africa (Harrison, 1958). Thus, the presence of Baetidae is no indication of clean water. Although we are dealing with a chemical effluent here, its earlier effects were similar to those of organic matter, e.g., lowering of oxygen content and encouragement of *Sphaerotilus* and *Chironomus*.

In 1956, scattered growths of sewage fungus were still present. By 1957, *Sphaerotilus* was found at the first two stations below the outfall only after careful search, several animals were still absent from the first station, and *Baetis* still showed a marked reduction in importance. The only other faunistic change that seems to have been associated with the effluent was that Ceratopogonidae (probably a species of *Bezzia)* was encouraged. The figure shows no changes in 1958 and 1959 that can be ascribed to the effluent. Thus, the river had returned to normal, and this process was easily followed by simple faunistic surveys. It is my contention that, in 1957, the presence of the effluent could not have been biologically demonstrated in any other manner; this even applies to the presence of *Sphaerotilus*, which can usually be found if it is specially sought, even in clean rivers.

Other differences between the surveys are apparent. *Baetis rhodani* was clearly much more important in 1957 and 1958 than in the other years. Very probably, this is because of the different months in which the surveys were made. The samples taken in March and April had large numbers, which probably was caused by a wintertime hatch of resting eggs. A similar explanation probably applies to the relative unimportance of *Rhithrogena* (mostly *semicolorata)* in the stations above the outfall in 1956. Further, there was a tendency in all years for the Ecdyonuridae to be absent or less abundant at the first station upstream of the outfall. This was, I believe, caused by a small, dirty stream and a small sewage works effluent that entered the river above this station. Neither produced any obvious effect, such as sewage fungus or diminution of oxygen, but they did contain suspended solids and so, presumably, rendered the substratum less suitable for flat mayfly nymphs. For other variations and irregularities (e.g., by Lumbriculidae), I can offer no tentative explanation, but it does seem that *Latelmis volkmari* was more common at the lower stations in all years. Probably, with more intensive sampling to reduce random error and more knowledge of the ecology of the organisms involved, satisfactory explanations would be forthcoming.

So far, we have considered only relatively mobile creatures that can readily reestablish themselves as soon as conditions become suitable. The limpet *(Ancylastrum fluviatile)* is far less mobile and is best recorded separately because it is less easily taken by net. In 1956, this mollusk was only found at and below 5.9 miles from the outfall; in 1957, it had moved upstream to 4.7 miles; in 1958, it was present at 1.1 miles; and, in 1959, it was found at all stations. This animal, therefore, showed the same general effects as the other invertebrates, except that its recovery was more delayed. The alga *Lemanea (Sacheria)* presented a rather similar picture by being quite absent below the outfall in 1956 and 1957, present at 2 miles and below in 1958 and 1959, but more common there in the latter year. These relatively sessile organisms are, therefore, very sensitive indicators of past pollution, and they take a long while to return.

My second example concerns a paper mill that complainants had accused of causing growths of sewage fungus. I was asked to report on the state of the river, but it was during July 1960 when the mill had been closed for 2 weeks and all the *Sphaerotilus* was gone; only scattered debris remained in the river bed. Samples were collected in the same manner as before, but were much less thoroughly worked in that the animals were not counted but were recorded on a simple scale (P = 1 or 2 specimens; F, about 5; C, about 25, A, about 100; V, several hundred). The stations were above the outfall, and 50 to 100 yards, 200 to 300 yards, about 1/2 mile, and 1 mile below the outfall. The results, given in Table 1, clearly show that in July 1960, conditions were not normal at the first two stations below the outfall, as shown by the changes in the numbers of *Leuctra, Helmis, Esolus, Hydracarina,* and *Ancylastrum*, and had been more severe at 50 to 100 yards than 200 to 300 yards below the outfall as shown by the changes in the numbers of *Baetis* and *Ephemerella*. A similar study conducted in March confirmed that sewage fungus grew only in the first 3 to 400 yards below the outfall and that it formed a complete carpet for only about 200

Table 1. THE OCCURRENCE OF CERTAIN ANIMALS IN SAMPLES FROM A BRITISH RIVER RECEIVING EFFLUENT FROM A PAPER WORKS

Macro-invertebrates	July 1960					Mar. 1961				July 1961				
	Samples[a]					Samples				Samples				
	1	2	3	4	5	1	2	3	4	1	2	3	4	5
Nais	C[b]	F	C	C	F	A	A	V	A		V			
Erpobdella	P		P			P	P	P		P		P	P	
Gammarus	P				P	P	F			P		P	F	F
Baetis	V	C	V	V	V	A	P	A	A	V	F	V	V	V
Ephemerella	V	C	V	V	V					V	F	V	V	V
Ecdyonurus	F		P		P	F			C	F		P	F	F
Rhithrogena						C			C					
Leuctra	F		P	F	F	F		P	F	A		C	C	C
Amphinemura						A		F	C					
Isoperla						F		P	C					
Hydropsyche						C		P	F					
Rhyacophila						C		P	F	P		F	F	F
Helmis	C		P	C	C	F		P	P	F		F	F	P
Esolus	C		F	C	C	F	P	F	P	F		F	C	F
Tanypodinae	C	A	C	C	C	P	C	P	P	A	V	C	A	A
Orthocladiinae	V	V	A	V	V	C	A	A	A	A	V	C	A	A
Chironominae	F	P	F	F	C		C	C		F	A	C	F	F
Hydracarina	F			F	P	P		P	P	P		F	F	P
Ancylastrum	A			A	V	A		P	A	V	C	V	V	V

[a] Station 1 was above the outfall; 2, 50-100 yd below the outfall; 3, 200-300 yd below; 4, 1/2 mile below; and 5, 1 mile below. [b] P equals 1 or 2 specimens; F, about 5; C, about 25; A, about 100; and V, several hundred, in 10-minute collections made with a hand net.

yards. At that season, other invertebrates that are absent in the summer were missing from stations 2 and 3 (e.g., *Rhithrogena)* or missing from station 2 and reduced in importance at station 3 (e.g., *Amphinemura, Isoperla, Hydropsyche,* and *Rhyacophila).* By the following summer, a treatment plant had been installed; sewage fungus was rare and only to be found at the first station below the effluent when carefully sought. Several creatures still showed signs of the effects of the effluent, however, although often only quantitatively. Thus, *Gammarus, Ecdyonurus, Rhyacophila,* and Helmidae were not found; *Baetis, Ephemerella,* and *Ancylastrum* were relatively less common; and *Nais* and Chironomidae were more common at station 2 than elsewhere.

This example shows that a very simple study of the invertebrates can be used to determine the extent of pollution, even when it is no longer occurring (1960), and to check on recovery while it is being abated. Both of the simple investigations outlined gave positive results when the levels of pollution were so slight that there was no other evidence that damage to other interests, such as fishery or water supply, was occurring. Complaints concerned only the presence of drifting sewage fungus that was entangling fishermen's lines; even these referred only to the first year in both investigations, and would very possibly not have been made except that earlier both rivers had been much more seriously polluted.

My thesis is then, that study of the macroinvertebrates is a useful tool in the detection of very mild pollution, that the study enables possible danger spots to be located and recovery to be closely followed, and that it retains its sensitivity down to levels at which most other methods of study cease to be useful. I would also emphasize that the examples I have given are of extremely simple, almost crude, investigations. More thorough sampling and mathematical treatment would, I imagine, increase the sensitivity even more. Mathematical approaches to this type of data have been discussed by Allanson (1961), Gaufin et al. (1956), and Patrick et al. (1954). It may be that this line of inquiry merits further study; but, as I hope I have shown, even very simple methods can detect pollution so mild that it scarcely merits the name under any definition. In short, although I have worked in other continents and other latitudes from those in which Beck has worked, I find myself in complete agreement with almost everything he said at the first of these symposia (Beck, 1957).

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DISCUSSION

Dr. D.D. Stone of Manitoba described the results of a study by the Manitoba Fisheries Section of the Pollution of Lake Winnepeg by the Red River. Pollution sources are communities and industries along the Red River. The Red River valley above the lake is a great beet- and potato-producing area. Pollutional effects were described as mild. Lake Winnepeg is located above the city of Winnepeg.

Dr. Jack Dendy of Alabama Polytechnic Institute, Auburn, Alabama, described how wastes from insecticide plants and the runoff from agricultural areas sprayed with insecticides suddenly killed fish in streams and reservoirs in Alabama. He asked how one would go about evaluating effects of pollution of this type. Dr. Hynes described methods used in Great Britain of collecting as many species as possible during a given time. Dr. Ruth Patrick suggested using species diversity and numbers of individuals as was done in a survey of the Sacramento River, Keswick Reservoir (California), and vicinity. Catherwood diatometers were used in this survey.

Paper chromatography was also mentioned as a means of determining the presence of insecticides.

Mr. T.W. Beak (Ontario, Canada) described how the effects on the macrofauna had been used to trace a source of pollution in Lake Superior for 5 or 6 miles.

Dr. Marcel Huet (Belgium) stated it was fairly easy to compare polluted areas with unpolluted areas by sampling the riffles of a stream above and below sources of pollution. The need to present biological data by statistical methods that can be understood and analyzed by engineers was discussed. It was emphasized that biological results cannot always be expressed by mathematical formulas; reasonable judgment of biological effects is often needed.

Mr. Carlos Fetterolf (Michigan Water Resources Commission) believed that, as he gained experience, he could more effectively judge the effects of pollution by qualitative rather than time-consuming quantitative work and questioned whether quantitative data are after all very meaningful.

Dr. Hynes stated that when we investigate pollution, we often only assay some particular quality of the water such as temperature effects, the effects of low oxygen, etc.

Mr. John Wilson observed that engineers plot sag curves for streams based on dissolved oxygen, BOD, probability of low flows, stream flows, etc., by which they predict how far downstream damaging effects may occur during low-flow periods. He recommended that biologists quantitate their data in the same way so that predictions could be made on what would happen to the bottom fauna, fish, etc., downstream. This would be an oversimplification of an extremely complex problem, however, involving the influence on the aquatic environment of many little-known industrial pollutants. Although some engineers and other physical scientists recognize the complexity of the aquatic environment, they overlook possible environmental damage by pollutants, "passing by on the other side," and rely solely on the parameters mentioned above -- DO and BOD -- for water resource planning. Not until legislation develops will the biological-environmental approach be considered.

Dr. Hynes, emphasizing the lack of data on quantitative effects of pollution, asked who could say what biological effects a reduction from 8 to 7 ppm dissolved oxygen would create.

Mr. Surber expressed his conviction that although the use of quantitative sampling apparatus entailed considerable work, it was justified in determining not only present conditions but also background data from which in the future it could be determined whether conditions in a stream had actually deteriorated or improved.

Dr. Ruth Patrick discussed the difficulties of using such terms as "present," "common," "frequent," and "abundant" as quantitative terms; it is better to consider groups of organisms rather than individual species.

Dr. Hynes mentioned that because stoneflies were early emergers, often appearing during the winter, they might be missed in the count of species present if the stream were sampled only during the summer.

The danger of trying to do too much with one group of organisms, such as the diatoms, was pointed out. Microorganisms are generally more sensitive than the macroinvertebrates as indicators of pollution. On the other hand, generally, stoneflies are indicators of clean water and the tubificidae are indicators of organic pollution. Dr. B.R. Allanson pointed out the similarities between the fauna of Europe and that of South Africa, and the macroinvertebrates in South African trout streams and those in European trout streams. He described the faunal changes that occurred at different elevations above sea level.

Dr. Huet showed histograms - of the seasonal abundance of different types of macroinvertebrates in streams, with some data based on samples of macroinvertebrates collected with a net during 10- to 15-minute periods above and below a source of pollution. The organisms were classed as present (P), common (C), frequent (F), and abundant (A).

Mr. T.W. Beak stated that the square-foot sampler, if used in areas of uniform materials, gives a good measure of the effects of pollution; that the number of individuals can be just as meaningful as number of species; and that we should produce drawings and illustrations of biological samplings that could be understood and used by the engineers.

Dr. R.O. Brinkhurst (University of Liverpool, England) described the great variation he had found between different areas in the quantitative sampling of various bottom organisms, particularly tubificids.

Dr. W. Schmitz emphasized the utility of quantitative data in delimiting the zones of pollution in a stream. It is very important, he said, to collect chemical data concurrently with biological data.

Mr. W.M. Beck of Florida cautioned that the presence of tubificids was not always an indication of a polluted stream and humorously reported the collection in a healthy stream of an Ekman dredgefull of tubificids due to the presence of a dead opossum in the immediate vicinity. Mr. Surber reported circular red colonies of tubificids in small/= mouthbassbrood-stock ponds at a fish hatchery at spots where aquatic vegetation had been raked into piles the preceding fall and left to dry. The localized abundance of tubificids apparently was due to an accumulation of putrescible materials where the vegetation decayed during overwintering. He emphasized, however, that in pollution surveys, organic enrichment of habitats was usually characterized by the presence of tubificids in unusual numbers.

Dr. W. Rodhe pointed out the need for investigating the functional aspects of organisms. Each organism has a metabolism of its own and both biological and chemical methods are required to determine the organism's characteristics. In lakes, organisms are distributed vertically and there are differences in spatial distribution. When a lake is polluted with organic wastes, assimilation and dissimilation are going on. Plecoptera are not present in streams that have more assimilation than dissimilation.

Dr. Hynes wondered what would happen to the assimilation process in a stream if copper were suddenly added to the environment.

Dr. A.R. Gaufin expressed the opinion that we should become more familiar with the life histories and habits of aquatic organisms and know what their requirements are; most of us cannot even identify our species, let alone know their life histories, etc. He stated we are still in the taxonomic stage. Dr. W.D. Burbanck stated that the number of taxonomists is diminishing and suggested that we send materials for identification to the National Museum; if we take the total productivity at different trophic levels, we need to know with what species we are dealing. Dr. Schmitz called attention to studies of the effects of pollution on the oxygen production of weeds in streams. Such stream studies are complicated by the effects of tributaries. The streams of Great Britain are mostly of one type, but streams on the continent vary a great deal. He recommended that groups of organisms such as the diatoms be studied by specialists in that field.

Dr. Patrick stressed the need for data on energetics and for more information on succession in the blue-green algae; the use of artificial substrata needs to be perfected.

Mr. Beck pointed out that energy flow techniques in establishing ecosystems require a tremendous amount of time. Dr. Hynes described the distribution at high altitudes of organisms some of which cannot endure sediment on the stones upon which they live. Stoneflies, he said, are sensitive to lack of oxygen, and mollusks are sensitive to heavy metals. Tubificids were also described as sensitive to the presence of heavy metals.

Dr. Burbanck inquired whether we were all to become molecular biologists. The names of the genera or species that are indicator organisms should be put to use.

Dr. B.R. Allanson stated that South Africans know the species of the more important groups. By hard work, one can learn the species and often many of their requirements. Aquatic insects, he said, are most sensitive to pollution or temperature changes during molting stages.

Dr. Hynes described the resistance of insect eggs to pollution and how a Norwegian worker found that insects fly upstream before depositing their eggs. These eggs are collected with a screen of 120 meshes to the inch. Dr. Allanson stated that care is needed when macroinvertebrates are used to indicate pollution; elevation, temperature, and turbidity can affect their presence. Some groups of insects or families drop out in going downstream from the headwaters. Mild pollution may produce its greatest effects in the upper reaches of a stream. Ecological changes such as changes in water temperatures, increase in turbidity, or pollution may have considerable influence proceeding dowstream.

Dr. N.W. Britt, of Ohio State University, described changes from 1942 to 1962 in the distribution of the burrowing mayfly *Hexagenia limbata*, midgefly larvae, and oligochaetes in Lake Erie. South of Rattlesnake Island on Lake Erie, *Hexagenia limbata* decreased from a maximum of 493 per square meter in 1942, to 15 per square meter in 1960, and to zero in 1961 and 1962. As the *Hexagenia* decreased, certain midgefly larvae and oligochaetes increased; in 1962 when there were no burrowing mayflies, there were 1,150 midgefly larvae and 7,825 oligochaetes per square meter in the lake bottom. No mayflies were found in the deep water of Lake Erie where the dissolved-oxygen content was under 0.5 ppm.

Mr. John Wilson discussed Dr. Howard Odum's opinion that species diversity data are the best means of illustrating pollution effects. He described Dr. Odum's method of counting up to 1,000 organisms in samples and recording the species diversity within this 1,000 group.

Concurrent Informal Discussion

This meeting opened with Mr. Thomas Beak, Chairman, describing some of the methods he and other investigators have tried in using "aquatic organisms as indicators of water quality."

Other ideas came from numerous persons on methods used or contemplated for such studies. Suggestions such as standing crop, extent of species diversity, and maximum production (e.g., g fish/unit area) were offered to replace or add to the idea of indicator species. The point was made that apparently no organism is so restricted that it occurs only in "clean or dirty" water.

Bioassay was run up and down the gauntlet. The physiological condition of the test animals and the time of year were listed as important considerations. Transplanting of algae-protozoa or macroinvertebrate groups by transferring multiple-plate samplers of glass or masonite, respectively, would lend to laboratory bioassay studies. In this case necessary considerations include the similarity of the basic testing water to the water at the collection site. Studying a pollutant by testing its components separately or in simple combination was reported on favorably by several researchers.

Several comments were made concerning the limits of laboratory studies and the necessity of combining laboratory and field studies to give a complete picture. The relative value of short-term studies designed to get preliminary data and give quick answers, and long-term studies where the complete analyses are necessary were discussed. The importance of life history studies, including basic ecological relationships, was pointed out, especially as related to following the cycling of elements through an ecosystem.

Several suggestions regarding the methods of tackling pollution problems were discussed. Establishing a position of "expert biologist," who would decide the water quality limits allowable, was among these. Another was the idea of studying specific organisms in specific situations. Although some animals may serve as pollution indicators, many others will react differently to a pollutant depending on other environmental factors. A third method involved a threefold study, including the usual biological and chemical measurements at the polluted site, and an investigation of the operations at the industry or source of pollution.

In describing the effects of a polluting agent, the term "tolerance" is quite prevalent. A lengthy discussion centered around tolerance and its modifiers such as moderate, high, or non-. From the comments, it seemed agreeable and advisable to use this term specifically as follows: (e.g., "sp. A is tolerant of 200 ppm silt turbidity for up to 30 hours before feeding ceases" rather than the general "sp. A is moderately tolerant of silt turbidity"). The comment was made that much data are now available in the literature and in personal files that could be used to group aquatic organisms in relation to their specific tolerances for specific substances.

Several methods of expressing results were discussed. Two types of reports were favored. One form was the "slide rule" for the engineers and the other is designed for the biologically minded reader. Several investigators gave their opinions of subjective versus objective reporting.

Several additional remarks came out regarding the need for life history-taxonomy work in pollution studies. Researchers from Great Britian, Canada, and half-dozen states regarded the lack of students entering taxonomic work as a serious problem. Possible reasons given were lack of demand, lack of favorable financial support from sponsoring agencies, and lack of interest among some college faculties and administrators. It was pointed out that in North America, much of the toxonomic-type work is done in the museums, whereas in Great Britian, for example, taxonomic workers are more evenly dispersed. One solution offered would have each ecologist take a particular animal group and work on the requirements or limits of its individual member species, considering not only lethal limits, but also limits for such things as optimum growth, reproduction, etc.

The closing ideas were aimed at people who send specimens to taxonomists for identification. Three suggestions seemed especially notable: (1) Supply adequate ecological data with the specimens to aid the taxonomist in the identification and in his personal knowledge of the species, (2) expect to pay for his services, and (3) expect a delay between the time the specimens are sent and the time identifications are received.

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ORGANIC PESTICIDES
THEIR DETECTION, MEASUREMENT AND TOXICITY TO AQUATIC LIFE

Oliver B. Cope * *and Clarence H. Hoffmann,* † *Chairmen*

PESTICIDE-WILDLIFE RELATIONS

Oliver B. Cope

INTRODUCTION

The detection and measurement of organic pesticides and their toxicity to aquatic life constitute the fish-pesticide problem. Researchers primarily interested in fish and pesticides in nature must, however, include the whole biota in the scope of their interest.

Studies on pesticides and fish usually begin with bioassays to determine acute toxicities of chemicals to various species of fish. People working in this area, once their testing methods are standardized, are invariably intrigued with questions about the application of their results to animals in the field. This leads to inquiries into the influence of factors in the environment on toxicity, into what happens to the pesticide in the environment and in the fish and other animals, into the nature and importance of chronic effects, and into many other consequences of field applications. To study these mechanisms, the researcher must concern himself with the detection, measurement, and toxicity of the pesticides.

DETECTION

Detection and measurement usually go hand in hand, but there are instances in which detection and not measurement is accomplished. Sometimes this is because the worker desires only qualitative information, but usually it is either because there are no quantitative methods available, or because those available are too time-consuming or require equipment that is not available.

A case of detection without regard to measurement might be the determination of the presence or absence of enough toxicant to kill fish following a fish-eradication project in a lake. Here, the placing of fish in a live-car in the lake gives a measure of safety for planting; this is commonly done by fishery biologists who do not desire measurements of the amount of toxicant in the water.

A case of detection with only semiquantitative measurements might be the use of bioassay for residues of herbicides. Here, where seeds are incubated in the presence of unknown amounts of herbicide in water or soil, and the inhibition of root or stem growth is compared with a standard curve, there is variation in response from species to species of seed, and from herbicide to herbicide. The establishment of standard curves is sometimes difficult, and results must often be interpreted semiquantitatively. This is frequently the best that can be done, since methods of analysis by chemical techniques have not yet been devised for many herbicides.

MEASUREMENT

Why do we measure organic pesticides? The pesticide problem is complex and involves interrelationships among the pesticide, the water, the animal in which we are interested, other animals in the environment, plants, bottom sediments, and other substances. It involves also the dimensions of time and space. So, if we are to understand the total consequence of the pesticide treatment, we must study the chemical in relation to the components of the environment. It seems necessary, also, to understand changes, and here is where time and space become so significant. The changes in pesticides over periods of time, under the influence of heat, light, and chemicals in the surroundings, and of microorganisms, must be measured if we are to know or predict whether our animals are damaged or helped by the pesticide. The effects of space in concentrating or in diluting the pesticide after it leaves the airplane or after it reaches the water should be understood. These effects can be most efficiently studied by measurements of the amounts of pesticides at times and places designed to show how much can be available to an animal for each time and position in the habitat. The fate of the pesticide, once it enters the animal body, must also be studied. Whether it is stored or excreted, and whether in its original form or in a more or less toxic form, are things we wish to know, and we must usually measure to find out.

Thus, the measurement of pesticide quantity appears to be a vital requirement in a comprehensive pesticide-animal program of investigation.

When embarking on a program of measurement of organic pesticides in water, in fish, in invertebrates, in mud, or in aquatic vegetation, the investigator must exercise judgment in the approach to the study. He must know what analytical methods are available for the particular chemical in question.

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He should also know the cost of analyses if the work is to be done in a commercial laboratory, and whether equipment, knowledge, and experience are locally available if the work is to be done in his own laboratory. The objective of the work and the amount of precision desired are factors that enter into the choice of analytical methods. Some knowledge of the limitations of the various methods is valuable if there is a question of the need for measurement of the original pesticide, or of its metabolites, or both.

What methods are available for measuring organic pesticides? Two principal analytical approaches are used, biological and chemical. Bioassay is the basis for biological measurement of pesticide residues. Here, a standard dosage-mortality curve for a specific length of time of exposure is first established. Then the unknown material is given to the test organism, and the response is referred to the standard curve to learn the amount of unknown. With this method, a variety of animal species is used for insecticide measurements; guppies, houseflies, mosquitoes, and Daphnia are common test animals for detection of chemicals in water. Seeds of plants, such as cucumber, tomato, flax, and corn, are popular for measurement of unknown amounts of herbicides. Bioassay has limitations, but can be very useful for detection of pesticides that cannot be measured by chemical methods and for verification of values that can be measured by the chemist. It can also be useful in cases where chemical or physical methods do not distinguish among toxic and nontoxic metabolites of a pesticide.

The measurement of pesticides by chemical and physical methods is more widely practiced than is bioassay. A variety of methods is used in the chemistry laboratory; each has its advantages and limitations.

Photometry, including colorimetric and spectrophotometric procedures, and gas chromatography are widely used for analysis following extraction and purification of the pesticide. These methods are versatile and accurate; lack of sensitivity has been their chief disadvantage. Recently developed detectors for the gas chromatograph have, however, made this a powerful tool for residue analysis. Another method coming into wider use is paper chromatography and color development with suitable reagents. This is an extremely sensitive procedure, but is not yet as accurate as instrumental methods. The shortcomings of this method for the determination of chlorinated hydrocarbons arise mainly from the lack of a suitable device for the measurement of the color of the spots. Another method involves the use of radioisotopes to trace the paths of chemicals in the fish and in the environment. This method is accurate and sensitive and can be used with chromatographic procedures to reveal the breakdown products of pesticides as they change.

With these tools, the chemist is well equipped to measure small and large quantities of pesticides from many sources.

TOXICITY TO AQUATIC LIFE

Toxicity of organic pesticides to aquatic life will be understood only through a study of the whole environment. As we study toxicity in the laboratory and envisage the extension of results to the field, we are impressed with the multiplicity of factors that can alter toxicity. We realize that one of our first considerations is the toxicity of pesticides to food organisms. This toxicity must be understood so we can predict the destruction of food supplies or the transport of pesticides in food to higher animals with subsequent acute or chronic toxicity. We are also struck by the changes in toxicity caused by influences of individual and multiple forces in the environment, such as hardness, alkalinity, and the presence of particular ions, which often control the toxic properties of organic pesticides. Moreover, physical features such as silt loads, light penetration, and water movements can affect the toxicity of a pesticide. The nature of the environment, then, controls toxicity to aquatic life, and often to a high degree.

One of the important problems in studying toxicity to aquatic life is the interpretation of effects on groups of aquatic animals. If good design is practiced in experimental work, with tight control over variables, and adequate replication, the researcher has an excellent chance to better understand trends in toxicity. The careful worker who determines the amount of variation in response within a group of aquatic animals usually finds it to be great. It is important to measure and report variation, but even with this knowledge and with indications that numerous replications should be made, one is often led to working with individual animals. As toxicological work with aquatic animals becomes more and more involved, the problem of relating exposure to effects often becomes so complex that groups are unmanageable from the standpoint of space and cost. It is then that the chances for predictions of responses in whole populations are reduced, and the investigator in most laboratories is forced to use individual animals. With individual animals the biologist and the chemist can achieve a precision in perceiving relationships between changes in organs and systems not usually possible in work with small groups of animals.

Toxicity to aquatic life is a vast subject, and has been the subject of an increasing amount of study and reporting in the literature.

We were able to consider only a few facets of the subject in these discussions, but I am certain that all of us will be convinced that toxicity of organic pesticides to aquatic life is a complicated affair, and that the detection and measurement of these pesticides is an indispensable ingredient of any comprehensive program for solving the pesticide-wildlife problem.

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EFFECTS OF TIME AND TEMPERATURE ON THE TOXICITY OF HEPTACHLOR AND KEPONE TO REDEAR SUNFISH

W.R. Bridges *

INTRODUCTION

A study to determine the effects of time and temperature on the toxicity of heptachlor and kepone to redear sunfish, *Lepomis microlophus*, was conducted in our laboratory at Denver, Colorado, in the fall of 1961. These two chemicals were selected because they were important at the time in the imported fire ant eradication program in southeastern United States.

TEST METHODS AND MATERIALS

The effects of the toxicants on the fish at the various times and temperatures tested were measured by determination of the Median Effective Concentration (EC_{50}). The EC50 is the concentration of the toxicant in the water that causes 50 percent mortality of the fish under a given set of test conditions. The EC_{50} values were derived by (the method of) evaluating dose-effect experiments proposed by Litchfield and Wilcoxon (1949). After preliminary testing had established the approximate range of toxicity, 4 or 5 concentrations of the test chemical were prepared for each EC_{50} value determined, and 20 fish were exposed at each concentration for the desired length of time. Mortality occurring in each concentration was recorded, and the results were plotted on logarithmic-probability paper. By application of Litchfield and Wilcoxon's simplified method, the EC_{50} and its confidence limits were rapidly obtained. EC_{50} values for kepone were determined for 6, 12, 24, 48, and 96 hours exposure at temperatures of 45°, 55°, 65°, 75°, and 85°F. Values for heptachlor were determined for 6, 12, 24, 48, and 96 hours exposure at 75°, and for 24 hours exposure at 45°, 55°, 65°, 75°, and 85°F. Further testing of heptachlor to obtain results comparable with the complete range of the kepone tests was prevented because fish of he required size and quality could not be obtained.

The toxicity tests were conducted in glass jars hat contained 15 liters of reconstituted water. The water was prepared by adding appropriate amounts of salts to deionized water; the pH and methyl orange alkalinity of the test water measured 7.1 and 35 ppm, respectively.

Technical grades of kepone (90.7 percent) and eptachlor (72 percent) were used in the test. Fresh tock solutions were prepared each day the tests were tarted. The toxicants were dissolved in acetone, and he aliquots required to attain the desired concenrations were pipetted into the test jars. Concentraions were recorded as the weight of active ingredient in milligrams per liter of test water, and the results reported here are expressed in the same terms. No attempt was made to maintain a constant concentration of the toxicant or to renew the toxicant during the tests. The results, therefore, reflect the concentration present immediately after the application, not necessarily the concentration in the water during or at the end of the test.

The fish used in the tests were redear sunfish from the National Fish Hatchery in Santa Rosa, New Mexico. They were all from the same lot and ranged from 45 to 55 millimeters in total length and averaged 1 gram in weight. To help prevent the occurrence of parasitism and disease, the fish were treated with 1 ppm of acriflavin and 20 ppm of formalin for 24 hours within 1 or 2 days after delivery from the hatchery. The fish were held in outdoor recirculating tanks where the water was similar in chemical characteristics to the water used in toxicity tests. While in the outdoor tanks, the fish were fed a commercial fishfood. They were removed from the holding tanks 3 or 4 days before being tested and were placed in the indoor aquaria where water temperatures approximated those of the holding tanks. Water temperature in the outdoor holding tanks ranged from 60° to 70° F. The fish were slowly brought to the desired test temperature and held at this temperature 24 hours before the test. Water temperatures in both the conditioning aquaria and test jars were controlled with water baths that were equipped with refrigeration and heating units with temperature controls that permitted regulation of the temperature within ± 1° F.

Since the test concentrations were not renewed, only 5 fish were tested in each jar. The use of the ratio of 1 gram of fish per 3 liters of test suspension or solution materially reduces the chance of masking the toxic effects of a chemical due to inadequate volume of test solution for the weight of fish being tested.

Artificial aeration was not required because of the relatively low weight of fish used in each jar. Absorption of oxygen at the exposed water surface provided adequate amounts, even at the highest temperatures tested.

The tests were conducted over a period of 40 days. To detect possible changes in resistance of the fish to the toxicants and to assure that valid comparisons of results could be made, frequent checks were made during the course of the testing period to determine if EC_{50} values were different from those obtained in early preliminary tests. The results of the check tests established that the susceptibility of the fish to the toxicants did not change. The results, in addition to disclosing the effects of time and temperature, reflect the relative toxicity of kepone and heptachlor in the range where both were tested. In many instances the relative toxicity of a chemical is difficult to determine because changes in the resistance of the same lot of fish occur during the holding period, or different lots of the same species and size exhibit great differences in resistance under identical conditions. Comparing results of different toxicity tests can lead to an erroneous conclusion concerning the relative toxicity of a chemical. Heptachlor was not later tested to the same extent as kepone because subsequent lots of redear were either more resistant or susceptible than the fish used in the series of tests reported here.

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TEST RESULTS

The toxicity of kepone to redear sunfish is presented in Table 1. Over the ranges tested, variation in the length of the exposure period produced considerably more effects than did differences in temperature. For the periods tested, 6 through 96 hours, the toxicity of kepone averaged 7.1 times greater at 85° than at 45°, while at the temperatures tested, 45° through 85°, the toxicity for 96 hours averaged 34.0 times the toxicity at 6 hours. There was a tendency for the effects of time to be more pronounced at the lower temperatures, and the effects of temperatures to be greater for the shorter exposure periods.

Increases in temperature caused moderate increases in the toxicity of heptachlor (Table 2). The toxicity at 85° was 4.2 times the toxicity at 45° for 24 hours exposure, the only time tested at all temperatures. In contrast to the situation found with kepone, the toxicity of heptachlor was only slightly affected by the time factor (Table 3). Heptachlor was only 3.7 times more toxic at 96 hours than at 6 hours at 75°, the only temperature tested at all exposure periods. Figures 1 and 2 illustrate the relationships of the effects of time and temperature on the toxicity of the two chemicals.

Since the resistance of the fish did not change in the period in which the tests were conducted, the results indicate the relative toxicity of kepone and heptachlor at the times and temperatures where both were tested (6, 12, 24, 48, and 96 hours exposure at 75° and 24 hours exposure at 45°, 55°, 65°, 75°, and 85°). EC_{50} values for heptachlor ranged from 0.017 milligram per liter for 96 hours exposure at 75° to 0.092 milligram per liter for 24 hours.

Table 1. TOXICITY OF KEPONE TO REDEAR SUNFISH

Temperature, °F	6 hr			12 hr			24 hr			48 hr			96 hr		
		Confidence limits[a]			Confidence limits			Confidence limits			Confidence limits			Confidence limits	
	EC_{50}	Lower	Upper	EC_{50}	Lower	Upper	EC_{50}	Lower	Upper	EC_{50}	Lower	Upper	EC_{50}	Lower	Upper
45	5.6	4.6	6.8	2.8	2.3	3.4	0.62	0.57	0.68	0.27	0.24	0.30	0.14	0.13	0.15
55	4.4	3.9	5.0	2.1	1.9	2.4	0.54	0.49	0.59	0.21	0.19	0.23	0.096	0.087	0.105
65	2.0	1.8	2.2	0.70	0.63	0.78	0.34	0.31	0.37	0.13	0.12	0.14	0.064	0.059	0.070
75	1.3	1.2	1.4	0.50	0.46	0.54	0.24	0.22	0.26	0.090	0.084	0.096	0.044	0.041	0.047
85	0.68	0.62	0.74	0.23	0.21	0.25	0.12	0.11	0.13	0.051	0.047	0.056	0.029	0.026	0.032

[a] Median Effective Concentration (EC_{50}) with lower and upper confidence limits for the probability of the .05 level, (p = .05), expressed in milligrams of active ingredient per liter.

Table 2. TOXICITY OF HEPTACHLOR TO REDEAR SUNFISH FOR 24 HOURS EXPOSURE

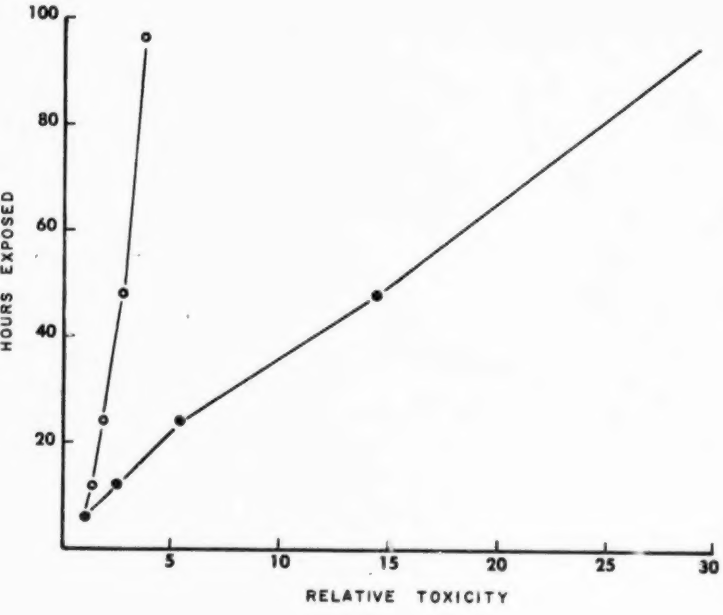
Temperature, °F		Confidence limits[a]	
	EC_{50}	Lower	Upper
45	0.092	0.085	0.099
55	0.064	0.058	0.071
65	0.047	0.043	0.051
75	0.034	0.031	0.037
85	0.022	0.020	0.024

[a] Median Effective Concentration (EC_{50}) with lower and upper confidence limits for the probability at the .05 level, (p = .05,) expressed in milligrams of active ingredient per liter.

Table 3. TOXICITY OF HEPTACHLOR TO REDEAR SUNFISH AT 75°F

Hours exposed		Confidence limits[a]	
	EC_{50}	Lower	Upper
6	0.062	0.057	0.068
12	0.046	0.042	0.050
24	0.034	0.031	0.037
48	0.023	0.021	0.025
96	0.017	0.015	0.019

[a] Median Effective Concentration (EC_{50}) with lower and upper confidence limits for the probability at the .05 level, (p = .05,) expressed in milligrams of active ingredient per liter.



igure 1. Comparison of the effects of time on the toxicity of kepone (solid circle) and heptachlor (open circle) at 75°F. Toxicity at the various times expressed as multiples of the toxicity for 6 hours.

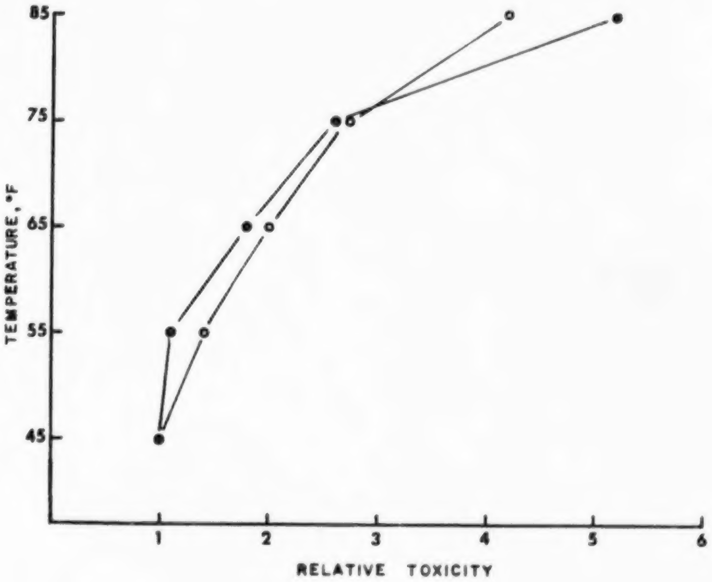


Figure 2. Comparison of the effects of temperature on the toxicity of kepone (solid circle) and heptachlor (open circle) for 24 hours exposure. Toxicity at the various temperatures expressed as multiples of the toxicity at 45°F.

xposure at 45°; values for kepone at comparable mes and temperatures were 0.044 and 0.62 milliram per liter. Considering only the times and emperatures where both chemicals were tested, the ighest toxicity for both compounds was recorded for ie 96-hour exposure at 75°. The lowest toxicity for eptachlor occurred at 24 hours at 45°. Kepone, howver, was the least toxic at 6 hours at 75°; the EC_{50} as 1.3 milligrams per liter. The differences in the ccurrence of the least toxic effects reflect differences the influence of the length of the exposure period the two chemicals. Heptachlor was more toxic an kepone, but the degree to which it was more xic ranged from 2.6 times greater for 96 hours posure at 75° to 21 times greater at 6 hours at 75°.

Only slight changes in concentrations of both kepone d heptachlor were required to produce different effects at a given time and temperature. The range in concentrations producing zero and 100 percent effects was quite narrow. In general, concentrations that killed all of the fish were only 2 to 2.5 times larger than concentrations that caused little apparent effects and no mortality.

ACKNOWLEDGMENTS

The author wishes to express his thanks to Austin K. Andrews, Terrence Cockrell, and Edward L. Davis for their assistance in conducting the toxicity tests.

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THE USE OF CARBON FOR MEASURING INSECTICIDES IN WATER SAMPLES

Charles C. Van Valin and Burton J. Kallman *

As use of organic pesticides has become widespread, considerable attention has been given to developing reliable and sensitive methods for the qualitiative and quantitative determination of these pesticides in water, plant and animal tissue, and soil. In our laboratory, paper chromatography and silver nitrate development of the purified sample provide sensitivity and accuracy excellently suited for rough quantitative work. With most of the common chlorinated hydrocarbon insecticides, a sample containing as little as 2 or 3 micrograms can be estimated with reasonable precision.

Water samples present peculiar problems. Liquid-liquid extraction of water can be used to provide suitable extracts from a reasonable sample size, but in our experience, the method is slow and often unreliable. Serious losses often occur perhaps due to adsorption or co-distillation. Additionally, in analyzing samples of extremely low concentrations, the volumes of sample and solvent become quite awkward to handle. In view of these drawbacks, and the additional difficulty and delay when shipping such samples from the field to the laboratory, we sought a better procedure that could be handled with relative ease by men in the field and that would provide reproducible results in the laboratory.

Rosen and Middleton (1955,1958) have described a method in which several thousand gallons of water are pumped through a bed of activated carbon to concentrate sufficient pesticide for infrared spectroscopy or colorimetric procedures. A somewhat similar process, but on a much smaller scale, has been described by Hancock and Laws (1955) for the removal of benzene hexachloride from water. Up to 5 liters of sample was passed through a 1-gram column of activated carbon. The carbon was then extracted with a mixture of glacial acetic acid and acetic anhydride, and a quantitative determination was made by the method of Schechter and Hornstein (1952).

During the early part of our work with charcoal adsorption, we used a similar column arrangement where a 1- to 5-gallon water sample was passed through a 10-gram column of activated carbon. We later found that yields were higher and more reproducible if the carbon was stirred into the water. Furthermore, stirring or shaking the carbon-water mixture at the time of collection in the field required less equipment and was less cumbersome and time-consuming than the use of columns.

PROCEDURE

Our procedure consists of four parts; adsorption, extraction, cleanup, and chromatography. To a 1- to 5-gallon sample of water is added 5 to 10 grams of activated carbon. The mixture is shaken or stirred for about a minute; the carbon is then removed by filtration on a Büchner funnel. The filter paper and carbon are transferred to a casserole, allowed to dry partially at room temperature, and then placed in a paper thimble and extracted in a Soxhlet extractor for 7 to 8 hours with 30 percent diethyl ether in petroleum ether. The solvent is then removed and the sample is ready for cleanup. The specifications on size of the sample, amount of carbon, time of stirring, and time of extraction are not intended to be limiting. Our results show that wide variations in these factors are tolerated with essentially no change in recoveries.

The most commonly used cleanup method in our laboratory is exposure to fuming sulfuric acid. The extract is taken up in *n*-hexane and shaken several times with 2 or 3 milliliter portions of a 50-50 mixture of concentrated sulfuric acid and 18 percent sulfuric acid. We removed traces of acid by passing the hexane phase through a column containing a small amount of anhydrous sodium sulfate. If additional cleanup is indicated, passing the extract through a 5-gram bed of MgO-celite (1:1,w:w), with petroleum ether-diethyl ether (94:6, v:v) as the eluting solvent, has also been used with success, although the procedure will degrade DDT. Where necessary, the acetonitrile-hexane partition (Jones and Riddick, 1952) may be used in addition to one of the above methods. An overall recovery of only about 60 percent in the partition step, as used here, makes its use less desirable, however.

A modification of the paper chromatographic method of Mitchell (1958) is used, with 2-phenoxyethanol as the immobile solvent and a silver nitrate solution as the chromogenic reagent. Our method has been described in some detail by Kallman et al. (1962). Aliquots of standard solutions of the pesticide are put on each chromatogram, and the values of the unknowns are visually estimated. These values are then corrected by application of average recovery factors.

A procedure recently tried, and still in a preliminary stage of development, involves washing the paper with a mixture of acetonitrile in water (1:3,v:v), placing proper aliquots of unknowns and standards on the paper, and washing again with the acetonitrile solution, followed by chromatography and development in the usual manner. This acts as a final cleanup step and may be useful in removing some persistent interferences. †

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† Robison W.H., personal communication.

RESULTS

The procedure outlined above was decided upon after varying a number of factors to determine the combination that gave the best reproducibility and recovery. Known amounts of various chlorinated hydrocarbons in organic solvents were added to deionized or tap water; the solutions were mixed and exposed to charcoal with shaking. Table 1 contains a list of factors that were varied and the limits between which no effects on recovery or reproducibility were found. The standard conditions were: 1 gallon of water, 50 micrograms of insecticide, 10 grams of charcoal (Nuchar C-190, 30 mesh), 1-minute shaking period at ambient temperature (ca. 25° C), immediate filtration, and extraction with 30 percent diethyl ether in petroleum ether. These factors were varied singly.

Table 1. RANGES OF VARIATION TESTED AND FOUND TO PRODUCE NO EFFECTS ON RECOVERY OR REPRODUCIBILITY

Variable	Limits	Insecticide tested
Amount of insecticide	10-500 ug	p,p'-DDT, heptachlor
Volume of water	1-5 gal	p,p'-DDT, heptachlor
Amount of charcoal	2-10 g/gal	p,p'-DDT
Time of shaking with charcoal	0.5-15 min	p,p'-DDT
Water temperature	20-33° C	heptachlor
Time lapse between shaking and filtration	5 min-60 h	p,p'-DDT
Percentage diethyl either in extracting solvent	20-50%	p,p'-DDT

Three factors were found to affect recoveries. One of these was the type of charcoal used. In addition to Nuchar C-190, we tried Cliffs Dow 10 x 30 activated carbon, which gave drastically reduced recoveries with heptachlor. Another factor was the manner of agitating the water-charcoal mixture. When 5-gallon samples were stirred vigorously with an electric stirring motor for 15 minutes in open containers, recoveries were reduced to less than half. Accordingly, such samples are now stirred manually for 1 minute, or the closed container is rolled or shaken for 1 minute. The manner of drying the charcoal following filtration is also of importance. The drying needed to permit efficient extraction should be done near room temperatures; lowered recoveries appear to result if drying is performed at elevated temperatures (ca. 40° C) in an air stream. Therefore, we merely allow the charcoal and filter papers to stand at room temperature until only slightly damp. There seems to be no advantage in allowing them to reach complete dryness.

Table 2 contains the results of all determinations made within the range of variations included in Table 1. These extracts were cleaned up by the sulfonation process alone.

DISCUSSION

The method described is suitable for rough quantitative determinations. It is being used currently in our laboratory for the analysis of water samples from various field operations and experimental stations. In some cases the water-charcoal mixtures are brought to the laboratory; in others, when filtration at the field site is feasible, the charcoal and filter papers alone are sent. The main advantage of the procedure, compared with liquid-liquid extraction, is the avoidance of the spurious low and negative results that were often obtained when water samples were sent to the laboratory in glass or plastic containers. An additional benefit is the savings in time. The liquid-liquid extraction of numbers of water samples requires much more actual working time than a charcoal method; this is especially true when large samples are used due to anticipated low concentrations of pesticide. Furthermore, except for the necessity of space on the Soxhlet apparatus, the charcoal method requires less laboratory space and less glassware.

One difficulty with this method that has not been mentioned is the presence of an artifact on the chromatograms. An extractable substance, apparently present on the unused charcoal in varying amounts, reacts with the chromogenic reagent and appears with an Rf identical to that of DDE in our chromatographic system. It does not interfere seriously with any of the insecticides with which we have worked to date. It is probable that a rigorous pretreatment will be necessary to remove this substance. Such a pretreatment would necessitate the redetermination of recovery factors.

Preliminary work with malathion, with procedures identical to those described above, indicate that the charcoal procedure can be used to determine this organophosphorus compound in water. The amounts of malathion extracted from charcoal were determined by fly bioassay, without cleanup. We are hopeful that the procedure can be applied to a wide range of insecticides, both chlorinated hydrocarbons and others.

Table 2. RECOVERY OF CHLORINATED HYDROCARBON INSECTICIDES FROM WATER WITH NUCHAR C-190

Insecticide	No. of determinations	Range	Percentage recovery	
			Median	Average
p,p'-DDT	33	37-60	45	46
heptachlor	21	50-93	73	72
heptachlor epoxide	7	70-92	83	80

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DISCUSSION—Part 1.

After a few specific questions on the relationship of the effects of time and temperature on the acute toxicity of heptachlor and kepone to redear sunfish the discussion soon turned to bioassay procedures and methods for the determination of "safe levels" of toxicant in the aquatic environment. The interest in acute and chronic toxicity of pesticides to aquatic life that was so evident in the second seminar was lacking. Throughout the seminar it appeared that there was much greater interest in chronic toxicity and the measurement and evaluation of sub-lethal effects than in acute toxicity, using death as the end-point. Some other end-points of laboratory evaluation of a pollutant that were suggested were histo-pathological effects, effects on protein metabolism, growth, and behavior. There was little discussion, however, of the significance of these sub-lethal effects on the ability of the test organism to survive, grow, and reproduce in the environment.

It was pointed out that there was much variation in published reports on the acute toxicity of pollutants to fish. Furthermore, it was suggested that there was great variation in toxicity results of a pollutant to fish even in the same laboratory. It was suggested that this variation in toxicity toward a species might be a laboratory artifact rather than a "real" variation. The laboratory toxicity of a pollutant is often greatly dependent on a multiplicity of physical, chemical, and biological factors.

A discussion was held regarding the use of a standard fish for bioassays and for basic physiological and biochemical studies. A standard fish would be a good basis of comparison of toxicity between various laboratories. It was pointed out that even though there was a standard fish, there still would be great variation in the environment of various laboratories. The guppy and bluegill were suggested as reference species. Water quality studies in any specific pollution study should be oriented toward the important species in that environment.

There was some disagreement with the methods of computing and expressing the results of toxicity tests. It was pointed out that the LD_{50} still occurs in the literature of fish toxicity, even though the toxicant is bioassayed in terms of concentration rather than a known dose. The term LC_{50} is the concentration of the toxic compound in the aqueous medium that will kill 50 percent of the animals exposed for a specified period of time. Furthermore, when lethality is measured, the term LC_{50} is more specific than the general term, EC_{50}, the concentration that, on the average, will affect 50 percent of the test animals.

Several participants seemed to be unaware of "Bioassay Methods for the Evaluation of Acute Toxicity of Industrial Wastes and Other Substances to Fish," as published in Standard Methods for the Examination of Water and Waste Water, which states that "Unlike the lethal concentration (LC) the term, 'tolerance limit,' is universally applicable in designating the effective level of any measurable lethal agent, including high and low temperature, pH, and the like. For this and other reasons, the term 'median tolerance limit' and the symbol, 'TL_m' are recommended."

In the laboratory determination of environmental requirements of aquatic life to serve as the basis of water quality criteria, the real pay-off is in the field. Bioassays can give only an indication of toxicity and of the physical, chemical, and biological factors affecting toxicity. In addition to laboratory studies ecological studies will be necessary for the determination of water quality criteria for pollution control.

HOW SHOULD AGRICULTURAL POLLUTANTS BE CONTROLLED?

C. H. Hoffmann *

To protect human health and fish and wildlife resources, there is currently much public concern about water pollution that may result from causes such as land-management practices leading to siltation of streams, improper treatment of industrial chemicals before discharge in waters, mine wastes, radioactive wastes, raw sewage, household detergents, and agricultural chemicals. These pollutants must be carefully considered in connection with the major beneficial uses of water; namely, for domestic consumption, agriculture, aquaculture, industry, recreation, navigation, power, and beauty of landscape. To maintain or restore the suitability of our waters for the above purposes requires a determination of water quality characteristics for each of these uses (Cottam and Tarzwell, 1960). Otherwise, it will not be possible to measure effectively water pollution, determine needed correction, or evaluate control measures properly. This paper will be limited to a consideration of silt, fertilizers, and pesticides as water pollutants.

NEED FOR AGRICULTURAL CHEMICALS

Agriculture is the United States' biggest business, employing more than seven million people on its 3-3/4 million farms and ranches. It is significant that these seven million produce the food and other commodities consumed by our 185 million people. Each farm worker today grows enough food and fiber for 27 people. A hundred years ago each farm worker produced enough food and fiber for only five people. Moreover, Americans are the best fed in the world and at a very reasonable cost. Agricultural chemicals have played an important part in the technological advances that have made this possible. Because of this great efficiency on the farm, much manpower is available for other pursuits.

Everyone is familiar with the great increase in yields of various crops attributable to the use of synthetic fertilizers. Each year additional farmers find that it is profitable to make greater use of fertilizers to produce an abundance of high quality crops. The increased use of fertilizers along with other improved practices makes it possible to produce more crops on smaller acreages than heretofore. This releases land, some of which is more desirable for forest and recreational purposes, and helps to alleviate certain siltation and agricultural chemical pollution problems.

There is no question about the importance and necessity of agricultural chemicals, including pesticides, in producing and conserving food, feed, and fiber and in protecting plants, animals, and man from serious diseases in this country. Sales of pesticidal chemicals, including insecticides, fungicides, rodenticides, and herbicides, at the basic manufacturers' level, amounted to $285 million in 1960 (Shepard *et al.*, 1961). The volume of pesticides required to control pests continues to increase each year and, along with fertilizers, is an integral part of our highly efficient agricultural production. Despite the widespread use of pesticides, however, losses due to pests cost this nation around 11 billion dollars annually. If pest control measures were not available, the losses each year would be several times this figure. Insecticides have provided outstanding control for many insects that damage or destroy fruits, vegetables, tobacco, sugar beets, sugarcane, cereals, cotton, pastures, ornamentals, and livestock. Many citizens are not aware of the hazards of pests in crop production; they are accustomed to buying almost any desired foodstuff of high quality at the chain store. If pesticides were banished, however, as has been suggested by some writers, many products would no longer be available, others would be of very poor quality, and the cost of foods would be higher.

In addition to controlling insects on growing crops, insecticides are used extensively to protect grain, foodstuffs, clothing, wood products, and many other materials from insect attack during transit and while in storage.

Insecticides have also been used widely to protect members of our Armed Forces from insect attack. Even with this help, however, malaria cases exceeded battle casualties in many theaters during World War II, for example, by 8 to 1 in New Guinea and 10 to 1 on Guadalcanal (Holway, 1962). The value of insecticides for the protection of civilians from insect attack is of even greater importance. It is estimated that during the first 10 years of use 5 million lives were saved and 100 million illnesses prevented through the use of DDT for controlling such diseases as malaria, typhus, and dysentery (Knipling, 1953). Of course, since that time millions of additional lives have been saved and additional hundreds of millions of illnesses prevented. Largely through the use of modern insecticides for mosquito control and the use of drugs, progress is being made to eradicate malaria on a world-wide basis. In the United States, from 1942 to 1953, approximately $54 million of Federal, State, and local funds were spent on the use of DDT and other control measures that resulted in the eradication of malaria that heretofore had been costing this Nation almost 10 times that amount annually in sickness and loss of production (Fritz and Johnson, 1960). Writers who condemn the use of insecticides forget that malaria was a serious problem in the U. S. not too many years ago and that in recent years eastern encephalitis transmitted by mosquitoes killed about a dozen persons in an outbreak in New Jersey. Insecticides have also been used extensively for the control of nuisance mosquitoes, biting flies, ticks, and chiggers. This permits farmers and livestock men to work in comfort and people to enjoy living in suburban areas and pursue recreational activities around fresh and salt waters.

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Forests must be protected from insects, diseases, rodents, and other pests if we are to have (1) lumber and other wood products; (2) clean water for irrigation, hydroelectric power, industrial, and domestic water needs; (3) meadows and grasslands for grazing domestic livestock and wildlife; (4) suitable habitats for fish and wildlife; and (5) scenic areas for camping, vacationing, and recreation. Some 30 million Americans spent nearly $4 billion in about 658 million days of hunting and fishing in 1960 (National Academy of Sciences, 1962). To protect these values, millions of acres of forest land have been successfully sprayed with DDT for the control of widespread outbreaks of defoliating insects. Many apparently well-meaning and conscientious conservationists have expressed opposition to such programs. At the same time I wonder if they have weighed the generally minor and temporarily adverse effects on fish, wildlife, and their food organisms that such treatments might cause against the substantial damage that usually occurs to wildlife habitats when insect outbreaks run their course. The outbreaks are often followed by devastating fires and, as a consequence, loss of timber, animal habitats, fish and wildlife, and undesirable water pollution.

Herbicides are used extensively for the control of weeds that compete with agricultural crops, undesirable brush, and aquatic weeds. The conversion of brushland and weed-infested lands to grass can increase the availability of water for livestock and irrigation and the amount of water stored in the soil. Aquatic weeds substantially reduce the velocity of water flow in irrigation and drainage canals increasing siltation and reducing carrying capacity of waterways. Aquatic weeds also reduce recreational values of waters by interfering with fishing, swimming, boating, and navigation. Moreover, the decaying organic matter produced by aquatic weeds causes off flavors in potable waters.

These examples are representative of many ways in which agricultural chemicals are used to man's advantage. These benefits include unrecognized improvements in recreational and aesthetic values. At the same time, however, adverse effects have resulted from their use, including the pollution of water.

STATUS OF AGRICULTURAL POLLUTANTS

Silt.--One of the great problems today is to increase the efficiency of water use and promote water conservation by improving soil and water management practices. Silt from eroding agricultural lands, denuded forests, and stream banks may be deposited in reservoirs, in stream channels, on flood plains, or in municipal areas. For example, the annual silt load carried by the Potomac River is estimated to be 40 million cubic feet, that of the Mississippi Basin above the delta about 500 million tons per year. This loss of soil represents important economic losses and at the same time pollutes water. Dissolved salts, suspended sediment, and turbidity are recognized as impairments to water quality. Accumulation of salts and toxic materials in soils is one of the hazards of irrigated agriculture. The leaching of accumulated salts in irrigated soils with water of low salt content may increase dissolved solids in return flow and thus aggravate water problems for other users. Moreover, muddy or turbid waters can have a detrimental effect upon fish and other aquatic animals. A contributing factor has been the demolition of forested areas, exposing the raw land to erosion, to take care of our expanded population needs in the form of new housing developments, industries, and super highways. Over the years many constructive measures have been undertaken to preserve the soil and to prevent siltation in waterways. Gradually the grassy prairies that were temporarily farmed have been restored to a permanent grass cover, some of which can be grazed.

Soil scientists have surveyed more than 700 million acres of land. They have classified and mapped each kind of soil according to its capabilities and management needs for the production of field crops, grasses, and trees. Today there are over 2,900 soil conservation districts that furnish information and help. Moreover, over a million farmers share costs with the government (more than $400 million in 1961) in carrying out conservation practices on about 340 million acres. Such extensive activities will contribute much to the reduction of siltation as a water pollutant.

Fertilizers.--Some fertilizers are bound to reach our rivers and water storages when you consider the many tons utilized in the cities and on the farm. In 1959, some 25 million tons were used on farms alone. Fertilizers find their way into natural water mostly during runoff and from soil leachings. Under certain conditions solutes from unusually heavy fertilizer applications on agricultural land may reduce water quality. On the other hand, small amounts entering farm ponds, lakes, and rivers are beneficial in increasing the numbers of plankton and other life that lead to better fish production. Thus far it appears that the important pollutional effect of fertilizers is the addition of nutrients to the water environment. According to a recent report of the U. S. Public Health Service (1962), "These added nutrients (fertilizers) are capable of supporting and often do support heavy aquatic growths, bacterial, algal, vegetative, and others, particularly in impoundments. Many of these forms of aquatic growths improve the fishing environment, but usually this proves to be a more serious detriment than benefit because such growths often cause serious tastes and odors in water supplies that are difficult and expensive to remove. Stream pollution from agricultural fertilizers, supplemented by the increasing amounts of nutrients from municipal and industrial wastes, is becoming a water pollution problem that may reach national significance. A widespread increase in the growth of nuisance organisms and plants would have serious degrading effects on water quality and use."

Pesticides.--The use of various pesticides for the control of insects, diseases, nematodes, rough fish, weeds, aquatic vegetation, brush, unwanted birds, and rodents varies from year to year, but the total manufactured output exceeds 500 million pounds. Many of these materials are applied by ground equipment and aircraft, and some of the chemicals reach our watercourses. They may be applied directly to the water, drift into water during the treatment of adjacent farms or forest, or washed in if soil erodes from agricultural land that has been sprayed. Only fragmentary data are available to point up the true effects of these materials as water pollutants.

It is estimated that about 50 million acres of agricultural land are treated with herbicides annually. When materials recommended for use on terrestrial areas are used adjacent to aquatic areas, there is some poisoning hazard to wildlife; however, most of the chemicals can be used for recommended purposes and at recommended dosages without serious immediate effects on wildlife or food-chain organisms (George, 1960b). Those chemicals used directly for the control of aquatic weeds and any that might leach from agricultural lands would become water pollutants.

The total amount of insecticides (active ingredients) used in an average year in the United States is estimated to be 225 million pounds. Estimates also indicate that less than 5 percent of the acreage of the 48 states, exclusive of Alaska and Hawaii, have insecticides applied on them in an average year; and only .41 percent of the total area generally considered favorable to wildlife, such as forest, grassland pastures, and wildland have insecticides applied on them in an average year (Hall, 1962). Moreover, recent estimates show that 85 percent of the acreage planted by our farmers and ranchers to crops each year is not treated with insecticides (Hall, 1962).

Following the release of DDT for civilian use, a number of research studies were conducted to determine its effects on terrestrial and aquatic insects and fish and wildlife in order that recommendations might be made to utilize the insecticide for control of destructive insects with minimum effects on other life. It was soon determined that effects were greatest on aquatic species, particularly bottom insects that serve as fish food. The results of studies with DDT and many other synthetic organic insecticides have been summarized by a number of workers including Rudd and Genelly (1956), Hoffmann (1960), and George (1960a). In general, on the basis of studies conducted by many individuals over the past 17 years, the contamination of ponds, lakes, streams, and rivers with pesticides that has been harmful to fish and other aquatic life has been minor in relation to the large amounts used. In fact, several writers have concluded that agricultural chemicals are rather minor as water pollutants in comparison with industrial and domestic wastes and other well-known water pollutants.

With particular reference to wildlife, a special committee established by the National Academy of Sciences-National Research Council (1962a) commented as follows: "With the thousands of tons of pesticides that are used each year in the control of pests, it may be concluded that, to date, the impact on wildlife, although not disastrous, is just cause for concern. Considered from a broad point of view, the known impact of pesticide use on wildlife has been considerably less than that caused by other everyday activities of man; yet it is important and every effort should be made to keep losses to the minimum as the use of chemicals increases. Additional research is needed to evaluate the impact of pesticides on fish and wildlife." It is recognized that the occasional improper disposal of pesticidal wastes from manufacturing plants, the cleaning of spray equipment in or near ponds or streams, the use of heavy pesticide applications in eradication programs and experimental studies, and the spraying of agricultural crops and forests have caused water pollution and some losses of fish and other wildlife in limited areas. Fortunately, nature has been liberal in providing a high reproductive potential for wildlife species, and there has been a remarkably quick reestablishment of species where losses have been sustained. Actually millions of acres in this country are treated annually to control or eradicate dangerous insect pests without any substantial damage to other interests.

Although the record on the use of insecticides and other agricultural chemicals is good, there is no room for complacency. With the increased use of these materials, which is surely to be the case, a determined effort must be made through research to ascertain the full story in regard to effects of different materials on various plants and animals that serve as food for larger animals, methods to measure the amount of such materials that contaminate water resources, and particularly, ways to remove these foreign materials from our water supplies. With regard to water pollution in rivers, DDT has been recovered in amounts ranging from 0.001 to 0.02 ppm from the Detroit, Mississippi, Missouri, and Columbia Rivers. The amount of DDT recovered from municipal water that has been purified is too small to affect humans and actually constitutes a minor part of their exposure to the insecticide (Nicholson, 1959). Specialists in water pollution believe that when insecticides are not present in sufficient quantities to kill fish, they will not constitute a public health hazard in drinking water. Rotenone and toxaphene can be removed from water during the purification process by use of activated carbon.

Recently a study was made on the water quality and aquatic life in a 2.7-acre pond within a 40-acre watershed, devoted largely to peach production, where parathion and minor amounts of chlorinated hydrocarbon insecticides had been used for insect control previous to 1960. The total amount of parathion applied in the watershed during the 1960 season was approximately 152 pounds active ingredient. Before insecticide use in 1960, 1.90 ppm parathion was recovered from pond bottom mud but only 0.02 ppb from two water samples (Nicholson *et al.*, 1962). Apparently most of the parathion entered the pond adsorbed on soil during a period of accelerated erosion. Parathion (0.01 ppb) was present in water collected 4 months after the last application of insecticide in 1960. This amount and the residue in the mud did not appear to affect the fish, zooplankton, immature aquatic insects, or Oligochaeta populations. However, there had been a significant reduction in immature aquatic insect numbers due to adults being killed by exposure to parathion residues in the watershed.

This general review has pointed up some of the problems associated with soil and water use and efforts under way to control soil erosion and thus prevent water pollution by siltation and agricultural chemicals. There are also problems associated with the use of agricultural chemicals, particularly pesticides, and the remainder of this discussion will cover ways in which pesticides are presently controlled and also will suggest additional ways to help meet the problem of controlling agricultural pollutants.

LEGISLATIVE CONTROLS

Legislation has had a great influence on the safe use of agricultural chemicals. The first law became effective in 1910 and concerned the manufacturing, labeling, and sale of insecticides and fungicides. During the ensuing 37 years many chemicals were added and formulations became more complex. The Federal Insecticide, Fungicide, and Rodenticide Act of 1947, administered by the U. S. Department of Agriculture, was modified with broadened authority to include herbicides, rodenticides, sanitizers, and disinfectants. Registration of a label was required before a pesticide could be offered for shipment interstate. The label was required to include adequate precautionary information to protect the user and the general public. When applying for registration, the manufacturer of the pesticide is required to furnish pertinent information on the composition of the material, rate and timing of application, the precautionary measures to be observed, the amount of residue likely to remain when used as directed, and toxicological data in support of a petition for a tolerance. The facts are reviewed by specialists in the Department of Agriculture who also may consult with state experiment stations and private laboratories to make full use of all available information. The scope of the Act was enlarged again in 1959 to include nematocides, defoliants, desiccants, and plant growth regulators. Recently the Department's Pesticide Regulation Division has been given authority to regulate poisons and devices for the control of a variety of wildlife pests.

The Miller Pesticide Residue Amendment to the Federal Food, Drug, and Cosmetic Act of 1938, the so-called Miller Amendment, became effective in 1954 and is administered by the U. S. Department of Agriculture and the Federal Food and Drug Administration. This law requires the thorough pretesting of a pesticide chemical before it can be sold interstate. The manufacturer must provide detailed data to the Department to show how much residue, if any, will remain on a crop after application and must furnish data on the degree of toxicity of the pesticide to warm-blooded animals. The Food and Drug Administration establishes a tolerance or maximum amount of residue of a chemical that may legally remain in or on a raw agricultural commodity when it enters interstate commerce. This level is deemed safe for humans.

Laws and regulations in more than 40 states either duplicate the same Federal requirements within the state or establish similar requirements to protect the safety of consumers. State laws also require labeling to warn users of any possible hazards in using chemicals.

These Federal and state laws have done much to bring about a thorough study of chemicals, including extensive pretesting, before they are marketed. Moreover, the strict regulations regarding usage and amounts that may remain as residues have emphasized anew the need for applying only that amount of material required for pest control; this factor in itself has avoided much unnecessary contamination of the environment, including our waters.

The above restrictions by law have come about as a result of years of careful study, and this would seem to be the appropriate way to approach any difficult problem. In recent years, as a result of much adverse publicity on pesticides, some of it of the scare type, some state legislatures have held special hearings on the pros and cons of the danger of pesticide use. Such hearings have been held in the states of California, Michigan, and Wisconsin and many specialists have been called to testify. To date, however, in view of the testimony received, they have not to my knowledge found it necessary to pass any additional laws to control the use of pesticides.

SUPERVISED PEST CONTROL

The action taken to control agricultural pests varies widely. By and large this action is an individual responsibility, determined by the producers of crops. Their decisions are influenced greatly by different advisors such as agricultural extension service agents who furnish current information on the control recommendations developed by state and Federal research agencies. Depending upon the problem the control action may be undertaken by the grower or he may contract to have the job done. In any event, the grower bears a heavy responsibility to see that the pesticide selected to control a particular pest is used according to the directions on the insecticide container, including all precautions, to assure safety to the spray operator; that the residue tolerance is not exceeded; and that the chemical does not drift or otherwise adversely affect his neighbor or other resources. In some states considerable emphasis is placed on different kinds of supervised control where growers seek the advice and services of pest control specialists. Often they will contract with airplane applicators well-versed in applying pesticides for particular jobs. Many states require the licensing of pest control operators, thus assuring that they are capable of undertaking certain kinds of pest control and that they have the proper equipment to make applications with safety to all. These precautionary measures can greatly alleviate drift of pesticides; however, the problem of drift beyond the control of operators is one of growing concern and more attention needs to be given to research on materials, equipment, and operational procedures to reduce this hazard.

Frequently community action is required for the control of pests. For example, in the case of mosquitoes, many counties or states have set up mosquito abatement districts with full-time officials who keep abreast of local problems and utilize currently recommended control methods, including insecticides, when required. Such an organized effort results in a more effective program and the use of less material than if individuals attempted to protect themselves by making repeated applications of insecticides.

There are, of course, many pest problems that are of sufficient magnitude to require joint Federal-State action. Large-scale eradication programs are undertaken whenever it appears that there is a good chance to get rid of a pest rather than to use chemical treatments year after year. Such programs have proved feasible for some pests and have certainly avoided the use of large quantities of pest control materials over a large area each year, thus reducing the opportunities for water contamination. Such programs are conducted under close supervision of personnel who are thoroughly trained in pest control activities and who are familiar with the principles of pest control as outlined in training and operational manuals. Such programs are often condemned by the uninformed public without weighing the alternative. Programs designed to eradicate new pests when first established in a restricted area could prevent the use of millions of pounds of insecticides that would be required annually if the pest becomes firmly established and spreads throughout its range of capability. Let's consider the cotton boll weevil. Forty percent of all insecticides used on agricultural crops are applied to control this pest. Drastic measures to eliminate such a pest when first introduced and established in a restricted area would be justified to avoid possible extensive annual use of chemicals thereafter on large areas. Most of the insecticides used today are to combat introduced insect pests.

FEDERAL AND STATE PEST CONTROL REVIEW BOARDS

In September 1961 a Federal Pest Control Review Board was established in response to President Kennedy's special message to the Congress on natural resources in which he stressed the need for coordinated efforts on the part of Federal agencies engaged in pest control programs to insure that all such programs will properly serve national interests, including public health, agriculture, and wildlife conservation. The Board consists of eight members, two each being designated by the Secretaries of the Federal Departments of Agriculture; Defense; Health, Education, and Welfare; and Interior. Recently the Board concurred with the U. S. Department of Agriculture in their plans for the use of insecticides and in procedures for the 1962 Federal-State program to eradicate the imported fire ant. The Board also approved plans developed by the Forest Service and cooperating agencies to control destructive insects and diseases on the Nation's forest lands. This approach evaluates the many factors involved in widespread control and eradication programs before they are undertaken and helps resolve any conflicts of interest. The Board has an opportunity to scrutinize any proposals for using highly toxic pesticides in Federal-State pest control activities, and if in their opinion a material is too hazardous from the viewpoint of health, wildlife, or water pollution, they can veto its use. Weighing such factors against overall benefits to be derived from the use of pesticides places a heavy responsibility on the Board members; however, deliberate consideration of all factors by informed individuals is an important step to resolve conflicting responsibilities.

At least one state has established a pesticide board of scientists and administrators representing different disciplines to approve sizeable pesticide applications before the spraying is undertaken. Records are maintained including the time and place of spraying, the materials used, and weather conditions. Some states require the licensing of pest control operators and the submitting of reports containing certain information on the pesticide formulation used to control a specific pest, the acreage involved, how the spray was applied, and other details. Such a history occasionally proves helpful if damage claims are made later and the pesticide is suspect. Moreover, a requirement of filing papers on intent to spray with certain materials would provide officials an opportunity to approve or deny the request.

In January 1961 a National Mosquito Control-Fish and Wildlife Management Coordination Committee was established consisting of members representative of important government and private agencies. This committee feels that individuals and groups can achieve coordinated control in the following ways:

"1. Promote mutual understanding of the problems and methods involved in mosquito control and in wildlife conservation.

2. Standardized procedures in the fields of both mosquito control and wildlife conservation.

3. Cooperate on legislative and other matters, which may involve rights-of-way, ownership, responsibilities, and administrative authority.

4. Share the responsibility of educating and disseminating facts to the public.

5. Mutually work out plans to hold and further develop public confidence in and support of joint mosquito control and fish and wildlife management projects.

6. Mutually stimulate, sponsor, and/or endorse research.

7. Cooperatively encourage demonstration areas for control of mosquitoes by methods not detrimental to fish and wildlife resources. And, vice versa, for management of fish and wildlife by methods not conducive to mosquito production."

REVIEWS OF PESTICIDE USAGE IN RELATION TO WILDLIFE AND WATER POLLUTION

I am convinced that studies and reviews of pesticide problems, usage, and research needs by experts serving on national committees and that forums to discuss this subject by private and public agencies have a beneficial effect in crystallizing scientific and public opinion. Science is moving forward so rapidly that the public becomes confused about how and under what circumstances new pesticides may be used effectively and safely. More and more there will be need to explain the results of research and to emphasize specific recommendations on the safe use of pesticides against each pest species. The results of committee studies presented in lay language will be of great use to the public in bringing current problems and methods of control into proper focus. In fact, the recommendations on pesticides should be given careful consideration by all of us for we are all affected in one way or another by pests as well as by pesticide usage.

An example of special reports of national interest are those recently published by an overall Committee on Pest Control and Wildlife Relationships established by the National Academy of Sciences-National Research Council (1962b). In addition to laying down the principles that need to be considered before undertaking a pest control program, one of the subcommittees has listed the precautions that should be followed to attain effective and economical pest control with minimum harm to wildlife. These same precautions should be followed to avoid or minimize water pollution, namely:

"1. Do not apply pesticides unless it is definitely established that chemical control is clearly the method of choice.

2. Do not apply unless the pesticide is registered for the purpose intended and directions for its safe use are clearly understood.

3. Do not apply unless the selected chemical, its dosage, formulation, and manner of application are known to be effective against the pest and least harmful to wildlife.

4. Do not apply in a large-scale program until both the chemical and the method of application are thoroughly field tested.

5. Do not apply on more than the minimum area required for control.

6. Do not apply in quantities greater than necessary to meet the control objective.

7. Do not apply without adequate supervision to insure that all precautionary measures are taken."

A number of organizations have held symposiums or meetings to review the status of pesticide programs, to point out some of the limitations as well as benefits, and to stress research needs. Special reference might be made to those held by the National Academy of Science, the New York Conservation Foundation, the Entomological Society of America, the North American Wildlife Conference, the Agricultural Research Service of the U. S. Department of Agriculture, the National Wildlife Federation, the Society of American Foresters, the National Agricultural Chemicals Association, the National Mosquito Control Association, the Soil Conservation Society of America, and these periodic seminars on biological problems in water pollution held by the U. S. Public Health Service in Cincinnati. More and more these groups are equating differences of opinion and conflicts of interest. The ultimate aim is to utilize pesticides for the maximum benefit of man with minimum damage to other organisms and minimum soil and water pollution.

EDUCATION AND PUBLIC RELATIONS

One of the best ways to control pesticides and other agricultural chemical pollutants is to educate the public on the use of these materials, emphasizing the need to use only the chemicals and amounts recommended for specific uses and required in good agricultural or pest control practice. Many citizens, particularly urbanites, are unaware of the need for chemicals to protect crops from pests so an abundance of high quality food is always available and so our Nation is protected against undesirable animals and from pests that damage, annoy, or transmit diseases to humans, livestock, and plants. At the same time, they need to be informed that there are certain risks involved in the use of chemicals and that adequate precautions must be taken to protect human life, property, beneficial insects, and wildlife and to avoid contamination of our water supplies. All information media available should be utilized to tell both sides of the story of the use of pesticides; they are a necessity and can be used with a high degree of safety if adequate precautions are taken at all times. Emphasis must be placed on the slogan "Read and Follow the Label" since this offers the greatest protection to the user and the consumer. Research workers have a responsibility to develop pesticide control recommendations that are not only efficient but also safe and that clearly state any precautions that must be followed. Extension workers, representatives of industry, and others have a responsibility to see that users are provided with the best materials to obtain control of specific pests with the least damage to other organisms. Those with broad experience in the pesticide field realize that there are certain conditions under which there will be a loss of fish and wildlife and beneficial insects. On the other hand, every effort should be made to counteract the scare story approach that alleges that pesticides upset the balance of nature everywhere (it has been upset ever since the Pilgrims settled in this country and to a degree by the Indians native to this country), destroy all birds, kill all the fish in the rivers, and basically cause cancer and other human diseases.

RESEARCH

Many of our problems on the control of agricultural chemicals as pollutants can be solved through properly orientated research programs on a sustained basis with adequate well-trained personnel and facilities. Speaking specifically on insect control, a strong basic research program would contribute much to practical control procedures irrespective of the methods used. These needs include studies on taxonomy, biology, nutrition, physiology, and biochemistry of insects. Pleas have been made by those opposed to the use of insecticides for the development of specific methods of insect control. Some have emphasized natural control, feeling that nature alone will adequately control most of the destructive pests. As valuable as natural control agents are in helping to regulate insect populations, the fallacy that they alone can meet the problem must be corrected in the minds of people, especially when the destructive insect involves accidentally introduced pests. There are many unexplored opportunities to use biological controls involving parasites, predators, and insect diseases to control insects, but the public must be prepared to underwrite an adequate research program to develop effective and dependable biological control agents. Eventually the user may also have to pay more for insect control. The development and use of biological control agents, especially those that are specific against one insect, would be in decided contrast to the development and use of modern wide-spectrum insecticides that, with slight modifications in formulations and methods of use, have been developed to control a large number of destructive insects. Industry has supported most of the basic development of insecticides in use today. Public supported institutions have contributed by determining in most cases how and under what circumstances they can be used for insect control. It is unlikely that industry can be expected to bear the cost of pioneering the development of new ways to use biological agents because of the uncertainty of a reasonable profit.

Other ways to control insects are under investigation, but most of the cost for such research must also be borne by public agencies. For example, the development of plant varieties resistant to insect attack is a most efficient and desirable way to control insects, but it takes 10 to 15 years, or longer, to develop each resistant variety. Because of the long time required and the generally urgent need for quicker solutions to insect problems, there has been only minor support for entomological research in this field. Certainly there are many possibilities for new approaches to insect control such as sterility by irradiation or chemosterilants, and some research is under way. The development and use of specific attractants, such as sex attractants, offers exciting possibilities to aid in detecting and controlling insects. Also, through research there are additional opportunities to find safer conventional insecticides or ways to use them to control specific pests, as in attractants. While research on long-range and possibly more desirable ways to control certain insects is underway, continued study of conventional insecticides is indicated because there are, as yet, relatively few suitable substitute methods available to meet the depredation of hundreds of species of insects that attack man and his agricultural crops, livestock, forests, granaries, and homes and that also transmit deseases that may be debilitating or fatal.

SUMMARY

There is no question about the importance and necessity of agricultural chemicals in producing and conserving food, feed, and fiber and in protecting plants, animals, and man from serious deseases. With the increased use of these chemicals, however, there is great concern about their presence in the environment, particularly as water pollutants. Recent studies of agricultural chemicals as water pollutants are reviewed to indicate the magnitude of the problem. There are several approaches to the control of agricultural pollutants, including Federal and state laws and regulations permitting the use of agricultural chemicals under specific conditions, supervised pest control programs in which trained and responsible operators use toxic materials, and the establishment of Federal or state pest control review boards made up of representatives of different agencies or interests to assure that widespread control programs are conducted in the public interest. The importance of educational activities and good public relations cannot be overemphasized in connection with the general safe use of agricultural chemicals to avoid unnecessary pollution. The interest of various national societies and other groups representing different disciplines concerned with various aspects of water pollution and the holding of seminars, symposia, and conferences to consider various problems are not only stimulating to research and control workers but the release of information associated with such meetings enables the public to become better informed. Moreover, some organizations help finance studies on special problems or seek support for research needed to answer urgent problems. The role of research in the discovery of new materials for pest control that are less toxic to other organisms and, therefore, of less consequence as water pollutants is emphasized. In addition, more research is needed on nonchemical or highly specific chemical methods of pest control that would obviate some water pollution problems.

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PESTICIDE POLLUTION STUDIES IN THE SOUTHEASTERN STATES*

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Present-day emphasis on chemical control of economic pests (approximately 92 million acres in the United States annually receiving pesticide applications)[1] has generated special concern among persons and agencies responsible for water pollution control and conservation. This concern has arisen because:

1. Pesticides are poisons to some animal or plant species, though their destructive qualities have great economic value when properly controlled.
2. Their effectiveness often demands widespread application where ultimate disposition cannot always be predetermined.
3. Their development has been so rapid that adverse side effects could not be completely assessed, and unfortunate incidents have attracted special attention and marshalled public opinion.

In brief, this concern over pesticides in general and insecticides specifically has its basis in fear. Fear results when insufficient knowledge is available to permit complete understanding of a problem, real or anticipated.

The history of pesticide pollution in water is relatively young. Reports of farm pond fish kills began appearing soon after 1945 when DDT and other organic insecticides became available to the general public. An awareness that these compounds might become serious water pollutants was created rather abruptly in the minds of pollution control officials in 1950 when extensive fish kills occurred almost simultaneously in 14 streams tributary to the Tennessee River in Alabama. Subsequent investigation showed that these kills were caused by insecticides washed from cotton fields following passage of a storm front with its attendant intense thunder showers.[2]

The thesis that insecticides could run off the soil with rain water was confirmed in 1954 by staff members of the Public Health Service working in cooperation with scientists from the U. S. Department of Agriculture.[3] Clay granules containing 11 percent dieldrin were applied on a measured grassy sod slope at the rate of 4.66 pounds of active ingredient per acre. Dieldrin was recovered in runoff from the first three significant rains after treatment in sufficient concentrations to kill fish.

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* Chemical analyses required in connection with these studies were performed under contract at Clemson College, Clemson, South Carolina, under the direction of Dr. H.J. Webb, Head of the Department of Agricultural Chemistry.

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These observations in the Southeast, reports of DDT recovered from major rivers of the United States such as the Mississippi, Missouri and Columbia, and public concern generated by the Imported Fire Ant Eradication Program of the U. S. Department of Agriculture stimulated the Public Health Service in 1959 to initiate a full-scale investigation of water pollution by pesticides. This project was established in Public Health Service Region IV with headquarters in Atlanta, Georgia.

The project's general purpose was to evaluate the occurrence of pesticides in both surface and ground waters to learn if use of pesticides was creating more than sporadic water pollution readily detectable by fish kills. Briefly, we wanted to learn how general was pesticide occurrence in surface and ground waters; what were their less obvious effects on organisms living in the aquatic environment; what factors relate to the presence or absence of selected pesticides in water; how well are they removed in the normal potable water supply treatment process; and what can be done about it? These questions have many ramifications involving multiple disciplines. The Pesticide Pollution Studies staff in Atlanta includes persons trained in chemistry, entomology, limnology, microbiology, and sanitary engineering. As the need arises we borrow the assistance of persons trained in other disciplines from agencies such as the Geological Survey, Fish and Wildlife Service, Tennessee Valley Authority, and various State agencies and municipalities.

The first of our studies was begun in June 1959 and continues in a 400-square-mile cotton farming area of Alabama drained by a single river system (Flint Creek). This study was planned to reveal and explain what happens in a natural agricultural environment. It is a living situation, real in every sense of the word, and, therefore, is not subject to the frequently heard criticism that it is contrived or artificial. Similarly, the scale of the study is sufficient to escape the charge that it represents only an isolated situation.

Agriculture is the only industry of importance in the Flint Creek watershed, and cotton is the only crop on which significant quantities of insecticides are applied. An estimated 15 to 16 thousand acres of cotton are grown annually, mostly on small plots averaging about 10 acres in size. Because the fields are so small, virtually no insecticide is applied from the air. This minimizes direct application to water surfaces.

On the basis of personal interviews with cotton farmers and subsequent statistical analyses, it was determined that 1959 usage of insecticide was approximately 86,000 pounds (technical). In 1960, a year of especially favorable growing conditions for cotton, usage was reduced to 59,000 pounds. Most popular insecticides were the chlorinated hydrocarbons, toxaphene (65% of total by weight of active ingredient), DDT (25%), and the gamma isomer of BHC (5%). These compounds were applied principally between July 1 and August 15. In 1959 approximately 57 percent of the total cotton acreage was treated one or more times whereas in 1960 only 34 percent was treated.

A municipality of approximately 5,500 people draws its water supply from Flint Creek. The stream at this point is fed by the entire 400-square-mile drainage system. Nearly continuous water sampling has been carried on at this water treatment plant since June 1959 by adsorption of insecticides on activated charcoal. Companion samples are collected for comparative purposes from raw and treated water. Sample size approximates 5,000 gallons and requires about a week to collect.

It has been determined that toxaphene and the gamma isomer of BHC occur in Flint Creek and pass through the municipal water treatment process undiminished in quantity. Toxaphene is present more or less year around with heaviest concentrations occurring in late summer during and just after the "cotton poisoning" season. Analyses are not sufficiently complete to permit statement of the seasonal trend for gamma BHC; however, that insecticide was present at least in the summers of 1959 and 1960.

The maximum quantity of toxaphene recovered was about 0.4 part per billion (ppb) while the maximum for gamma BHC was a little more than 0.75 ppb. The true maximums are unknown because of recognized limitations in the sampling and analytical processes, and it is assumed that quantities greater than this were present.

Sampling of the river and its tributaries showed that contribution of insecticides occurred from the watershed as a whole rather than from a few favorably located cotton fields. Further evidence indicated that these insecticides ran off the land surface dissolved in water.

DDT,which constituted 25 percent of total insecticide usage, has not been recovered. Its absence may relate to its strong affinity for organic matter in the soil and to its extreme relative insolubility in water (less than 1 ppb).

In 1959 and 1960 limnological investigations were conducted in Flint Creek to determine effects of insecticide presence on aquatic life. So far as was determined, bottom organisms and fish populations were not reduced in number or species composition. Nor was there a demonstrable effect on zooplankton although it must be added that numbers were too meager to permit critical study. Zooplankton populations of low order are a normal condition for turbid streams in the Southeast similar to Flint Creek.

A second study involved factors related to parathion contamination of a farm pond located in a peach orchard, persistence of that insecticide, and biological effects of that contamination.[4] It was determined that parathion contamination of water in the pond (0.02 ppb) and pond bottom mud (1.9 ppm) occurred in March 1960 during a period of accelerated soil erosion and before any use of parathion that year. The source of parathion was the acid orchard soil where the insecticide had persisted since the previous summer (at least 9 months). On the basis of a literature survey, this prolonged persistence changed our opinion of parathion that had been thought to decompose much more rapidly.

During the 1960 spraying season (April 11 to August 1), parathion concentration declined in the pond bottom soil, but increased in the water to a maximum of 1.2 ppb. Contamination during that time apparently resulted largely from windblown spray. No adverse effect on fish or other aquatic life was evident from prolonged exposure to these traces of parathion either in the water or underlying soil; however, critical suppression of chironomid midge larvae populations did occur because of reduced recruitment. The adult population was diminished by exposure to parathion spray in the terrestrial environment.

Not all pesticide pollution problems are a result of usage in agriculture. An industrial discharge of liquid wastes containing parathion caused an extensive fish kill throughout 25 miles of a receiving stream in 1961. This stream joined a river that flowed at that time at approximately 7,000 cfs. Fish were affected in portions of two major reservoirs of the river. Affected, but living, fish taken from the second reservoir about 100 river miles from the industry contained from 0.75 to 1.5 ppm of parathion.[5]

A start had been made toward evaluation of pesticides and their usage in relation to water pollution, but much remains to be done. It is evident that pesticide pollution can and does occur. Factors determining the degree of pollution are: (1) water solubility of the pesticide, (2) persistence in the soil, (3) quantity applied, (4) formulation, (5) method of application, (6) slope of the land, (7) soil characteristics, (8) volume and intensity of rainfall, and (9) soil conservation practices including degree and type of vegetative cover.

Occurrence in surface water of pesticides in quantities below the level acutely toxic to aquatic life may be rather common in areas of routine pesticide usage. The threshold below which no harm is caused has not been determined. The recognizable threshold, not necessarily identical with the true threshold, will depend entirely on the sensitivity of methods for detecting the effect.

Evaluation of effects of acute exposure is not particularly difficult and is fairly well known for some of the fishes, a few species of zooplankton and benthic organisms, as well as man. The evaluation of effects of chronic exposures at low levels is much more difficult. Much more work in this area is critically needed.

Pending more complete investigation of the possibility of chronic low level toxicity, certain actions may be desirable to minimize or avoid water pollution by pesticides. One action that may be desirable is improved control of aerial application of pesticides. This may take the form of revised State legislation where needed or other regulatory action to prevent incorrect or careless application of chemical toxicants and to assure adequate maintenance of equipment. Twenty-two percent of the total acreage sprayed or dusted in the United States in 1958 was treated by aircraft.[1] Aerial pesticide application is a frequent cause of acute fish poisoning. In many such cases improved skill in application or perhaps repair of leaky equipment may be all that is necessary to mitigate such losses.

A second desirable action is improved education of farmers, pest control operators, and the public about the consequences of misuse of pesticides. Such instances as cleaning a spray rig in the creek, "letting a little poison go into a lake to see what would happen," or similar thoughtless or careless acts too frequently have been causes of serious episodes.

A third action is continued efforts to promote and improve soil conservation practices, especially as they relate to retardation of surface water runoff. The Public Health Service studies have shown that transport of pesticides in surface runoff can be a significant means of surface water contamination.

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DISCUSSION—Part 2.

After a few specific questions on the report, "Pesticide Pollution Studies in the Southeastern States," the discussion centered on the philosophy of the use of pesticides. It was pointed out that the toxicological problems involved in the manufacture and uses of pesticides are great. The responsibility of the manufacturer ends with the sale of the product. Industry is responsible for the technical information. With many pesticides this does not include information on the toxicity to fish and wildlife. Where the pesticide is to be applied to water for aquatic weed control, toxicity information is developed for aquatic life.

It was pointed out that a little is known about the acute toxicity of some pesticides to a few aquatic species. There is a critical need, however, for water quality criteria based on long-term, continuous-flow studies to determine cumulative effects of pesticides that are neither lethal nor significantly harmful for survival, growth, and reproduction of aquatic life. Also more research is needed to determine the effects of pesticides transmitted in the food web.

Along with the critical need for water quality criteria for aquatic life, there is a great need for methods to detect and measure pesticides in water and in tissues of plant and animals.

It was indicated that more research was needed for the control of pests without chemicals. There are great possibilities in the field of biological control. There is a need for more basic research and ecological studies in this field.

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THE RELATION OF LAND USE TO THE AQUATIC ENVIRONMENT

W. E. Bullard, Jr. and A. D. Harrison,† Chairmen*

ROLE OF WATERSHED MANAGEMENT IN THE MAINTENANCE OF SUITABLE ENVIRONMENTS FOR AQUATIC LIFE

*W. E. Bullard, Jr.**

INTRODUCTION

Areas of land receiving surplus precipitation contribute surface runoff and subsurface seepage that form streams and lakes. Conditions on the land affect the amount, the timing, and the quality of the water reaching streams. It is the purpose of this paper to describe those characteristics of land, its cover, its use, its development, that modify the movement and quality of water, and what can be done about them through management. Specific emphasis is placed on factors affecting the aquatic habitat.

DEFINITIONS

"Watershed" may be defined as the land area contributing water by surface or immediate subsurface flow to a lake or to a specific point on a stream. Webster's New Collegiate Dictionary says "the whole region or area contributing to the supply of a river or lake; a drainage basin"; but we will not want to include areas producing groundwater that moves across surface divides to supply a stream, as occasionally happens.

"Watershed management" is defined in the U.S. Forest Service Manual, Title 2500, as "the protection, conservation, and use of the natural resources of a drainage basin to keep the soil mantle in place and make water available in a manner which best serves human requirements." It is further stated that watershed management is not a single operation but a complex of several activities. We might modify the definition to say "which best serves the aquatic environment," but I believe we will come to much the same result either way.

"Suitable environment for aquatic life" is perhaps more difficult to define. If we think of it in terms of the natural environment and natural range of conditions in which desirable aquatic species such as trout and salmon in the Northwest have developed, we can determine certain parameters of temperature, dissolved material, suspended material, and streamflow regimen. Suitable temperatures would cover the range within which the entire life cycle of desirable species and their food chains could be completed, and within which the organisms could maintain reasonable growth and reproduction rates. The same criteria would apply to dissolved material, both gas and solid, to suspended material, and to streamflow regimen. Thus, the "suitable aquatic environment" would not become too warm, nor too cold, nor change too rapidly; would not contain harmful loads of suspended material that would cut down light, or dissolved material that would be toxic, though it would contain sufficient nutrients to support growth and balance removals; and would not be subject to damaging flood flows nor to drying up in the low-water season.

CAUSES OF CHANGES IN ENVIRONMENT

Natural Factors

Numerous natural occurrences on the land have effects on the aquatic habitat. Earth movements, from slow gravity soil creep to sudden landslides and avalanches, add sediment and organic debris to watercourses, increase turbidity of streamflow, sometimes block stream channels, and occasionally cause damaging surges. Lightning sets fires that destroy the vegetation cover on watersheds and remove riparian shade from streambanks; after a fire, ash blows or is washed into streams to bring about drastic (though short-lived) chemical changes, denuded soil erodes readily and adds to sediment loads and turbidity, runoff is rapid and streamflow becomes flashy, and less water infiltrates to underground storage to maintain low-season flow. Major floods caused by excessively long-continued rainfall or rapid snowmelt tear up channel bottoms, destroy bottom biota, break down channel banks, add organic debris and sediments to stream loads, and pile up debris jams that block channels. Ice may be a significant factor; it interferes with gas and heat exchange, bottom ice dislocates bottom biota, surface ice forms jams and flood surges, and, influenced by wind, ice erodes streambanks and lakeshores.

There are natural factors other than physical. Animals may create situations on the land that directly affect water. Beavers build dams that make swamps, trap sediments, and add to the organic load of the water. Big game (elk, bear) wallow in the streams and increase turbidity; rangelands overgrazed by big game are readily eroded and cause flash runoff and increased sediment loads and turbidity. Ground squirrel burrows may cause gully erosion with similar consequences. Insect infestations on riparian vegetation seasonally add to the organic load of streams. Leaf fall from riparian deciduous trees and shrubs also adds to the dissolved organic load and to debris in the streams.

Development and Use Factors

Soil disturbance by road, pipeline, and powerline construction, by the timber harvest, by farming operations, or by heavy grazing use by domestic livestock increases erosion and sedimentation, increases runoff, decreases infiltration, and affects streamflow regimens. Denudation of the natural plant cover by fire, by heavy grazing, by the timber harvest, by clearing for construction, or for agriculture has similar effects; and the loss of shade increases stream temperatures and decreases riparian cover. Blocking streams by dams, diversions, culverts, etc., interferes with fish movement, changes stream temperatures, modifies or permits regulation of streamflow regimens, and creates storage space to trap sediment and debris as well as to hold water for increasing low-season flow. Stream channel changes by relocation for road construction, by encroachment of road fills, or by levees, lining, or diversions, destroy existing habitat and modify the streamflow regimens.

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Addition of materials other than sediments and natural organic debris to streams is a common consequence of land development and use. Animal wastes from corrals and feedlots and pastures may drain into streams to increase nutrient loads; it is normal practice in many areas to have corrals and feedlots along small streams, and the stock may wallow in the streams and break down the banks to increase turbidity and sediment loads as well as organic nutrient loads. Fertilizers used in farming are leached away to increase the nutrient load of streams. Insecticides, fungicides, and weedicides applied to soils and crops on farms, to forest and range areas, or to roadside strips, and chemicals used in fighting forest and range fires may wash into streams with serious and sometimes prolonged toxic effects. Machines used on the land and power boats on the water present a hazard of oil and gasoline contamination; the boats with underwater exhausts add further to the toxic load in the water with materials in the exhaust fumes. Cement and cement-curing chemicals or creosoted timbers used in dam and other construction on streams may add toxic materials to the dissolved load in streams. Mine drainage adds highly mineralized and often toxic discharges to streams. Dust-laying and surface-binding chemicals used on roads wash into streams to provide still further increases in the dissolved and often toxic load. Finally, the litter and garbage and sewage from farmsteads, work camps, and recreation areas often get into streams to increase debris and nutrient loads.

An index to the significance of these "additives" is given by the reports on fish kills for 1961. The deaths of some 5.6 million fish were attributed to agricultural pesticides, and 1.1 million dead fish to mining wastes, countrywide, for the areas reporting.* This is not the total for the country.

POSSIBLE CONTROLS

On Natural Factors

Though we are not yet to the point in weather modification where we can prevent the heavy rains or rapid snowmelt that cause great floods, or where we can conjure up rains in the dry season to maintain streamflow, there are many of the natural factors affecting streams and the aquatic habitat that we can modify and regulate. Wildfire can be suppressed and kept from running free to despoil vast watershed areas. Dams can be built for flood control, to trap sediment and debris, to store water for release to build up natural low flows, or to control temperature regimens by releases from various reservoir depths. Dams need not be necessarily harmful to the aquatic environment. Channel clearing and cleanup can remove debris jams that block or threaten to block streams, and reduce the bank-cutting and sedimentation hazard. Animal populations can be regulated; beavers can be trapped where they are not wanted and planted where they are wanted; special seasons and hunts can be established to reduce the numbers of bear, deer, and elk; rodents can be poisoned; and insect epidemic outbreaks can be treated with sprays. Though such control operations may themselves provide some hazard to water quality and the aquatic environment, the jobs can be done carefully and with a minimum of risk.

Regarding other natural factors, it is easier to avoid touching them off than to correct the results or control them. Road locations should be routed around areas of unstable soil and rock to avoid triggering slumps and slides. Channel relocations and diversions should be made with an eye to local topography and soil conditions so that they do not undercut steep slopes or result in excessive bank-cutting. Riparian hardwood trees and shrubs can be removed and replaced with conifers in many regions, or at least with species that present less of a leaf-drop problem and that are not tasty and attractive to tent-caterpillar colonies.

Sometimes corrective works are needed. Avalanches tend to repeat in the same paths year after year, but judicious placement of barriers and planting of trees at breakoff points can prevent them from starting. Undercut slopes can often be supported by toe walls; streambanks can be stabilized with revetments and vegetation or protected with wing walls. Heavy debris loads in streams can be stabilized with channel sills. Burned areas can be sown with grass or mustard to provide a quick-growing cover crop to hold the soil against erosion, and planted to shrubs and trees to get a permanent cover re-established rapidly.

On Development Factors

In farming operations, there are many possible controls. The adoption of soil-conserving cultivation practices, such as cultivation on contour, leaving contour strips uncultivated between cultivated areas, preparing grassed waterways, and avoiding long periods with the soil bare and open to wind and water erosion, reduces soil movement and stream sedimentation. The use of cover crops as a green manure for the soil, as well as to hold the soil against erosion, is another aid in prevention of turbidity and sedimentation in streams. Land drainage has destroyed many aquatic habitats, and drainage ditches have themselves been sources of sediment as well as carriers of eroded material from the land above to the streams below; but the ditches can be replaced by subsurface drains or by grassed waterways, and their gradients can be controlled to reduce erosion. Irrigation waste water may carry nutrients, toxic materials, and erosion sediments into streams; except on those alkaline soils where heavy irrigation is used to leach out excess salts, irrigation can be more efficient, by sprinkler, for example, rather than by flooding, to reduce the waste flow and the load carried.

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*Page 9A, June 1962 issue of Water and Sewage Works Journal

Road construction, perhaps the chief source of sediments in the Northwest, can be improved in every phase. Mapping out road locations to choose sites on benches away from stream channels, minimizing cuts and fills, and avoiding areas of unstable soil and rock will do much to reduce sediment contributions. It should also help reduce both construction and maintenance costs. In the construction itself, restricting the heavy soil-moving operations to dry weather and hauling excess material to safe disposal rather than direct overcasting to stream channels, including cut and fill slope stabilization as a final step in construction, and avoiding encroachment on channels, all will cut down sediment contributions and damage to the aquatic habitat. Drainage from the road itself as well as intercepted drainage should be turned off at short intervals to avoid building up erosive concentrations, and should be disposed where it can soak into the soil rather than led directly to stream channels. Road surfaces should be rocked and graded regularly to avoid rut formation and muddy drainage. Culverts should be installed on the natural stream gradient; on streams where fish passage is concerned, culvert types and methods of installation meeting state fish and game department requirements should be used. Culverts should extend well beyond the fill slopes and should be protected with both inlet and outlet headwalls to prevent erosion from undercutting the slopes. Cut and fill slope stabilization may require drains and toe walls and bulkheads, but generally may be accomplished by establishment of a dense vegetation cover of plant species adapted to the particular region. Maintenance of ditches and crossdrains, road surfaces, and cut and fill slopes should be done in such a manner that surplus soil and rock are not overcast directly into channels, or where they can erode into channels.

Timber harvest layout can prescribe cutting boundaries that leave protective strips to shade streams and to screen out debris that might otherwise roll or be washed into streams. Cutting units and the access road system need to be thoroughly planned in advance to take advantage of topography and to avoid critical soil situations. Felling timber should be done uphill away from channels, and yarding the timber should avoid crossing channels. Highlead uphill yarding or a skyline downhill yarding system will avoid soil disturbance. Tractor yarding should be restricted to gentle slopes and dry soils to avoid gouging and compaction, and skidtrails should be used only two or three times to avoid digging new drainage channels on the slopes. After the logging job is done, crossdrains should be installed in skidtrails and the soil should be seeded or planted to provide a cover against erosion. Landings should be located away from streams and diked to prevent the escape of muddy drainage. A clean and thorough harvesting job will reduce the need for fire for slash cleanup, and avoid ash and further erosion hazards. As with road construction, soil stabilization afterward should be considered and done as part of the logging job; skidtrails, landings, and temporary spur roads should be drained, seeded, mulched, and planted as the local situation indicates. Throughout the entire logging and cleanup operation, care must be taken to avoid stream contamination from oil and gasoline, whether by accidental spills or from equipment operating in streams; trucks and tractors should be kept out of streams and off stream banks as much as possible to avoid breaking down the banks and tearing up the channel as well as to avoid direct contamination. Channel crossings should be made on temporary culverts that can be installed and later removed with a minimum of channel and bank disturbance.

Grazing by domestic stock on the open range can be restricted in numbers of stock and season of use to avoid too great decrease in density of the plant cover and to prevent excessive soil compaction. This will help maintain infiltration rates, reduce runoff, and decrease erosion hazards and sediment contributions to streams. Stock should be fenced out of streams, and watering places should be so developed that they are not fouled and muddied by trampling. This will avoid breaking down stream banks and consequent sedimentation and turbidity. Dead animals should be disposed by burial or burning away from streams.

In pipeline and powerline construction, much the same rules should be followed as in road construction. Adequate drainage with safe disposal of drainage waters is needed for all areas of disturbed soil to avoid erosion and production of sediments. Disturbed soil should be stabilized by seeding, mulching, and planting after the construction job is finished. Provision must be made for safeguards against accidental spills or leaks of oil or other contaminating materials that might escape to streams.

Mine drainage should be led to disposal by infiltration into the soil rather than allowed to move directly to stream channels. Mine dumps and spoil piles should be located where they will not erode into channels, and should be stabilized with a vegetation cover to keep them from eroding. Waste waters should be ponded to trap sediment before the waters are returned to streams. Gravel and road material should be taken from quarries away from streams wherever possible, and wash waters should be ponded before release. Where removal of gravel from streams is necessary, it should be done at seasons that will not interfere with spawning and hatching of fish, and at water stages and by methods that will minimize sediment production and damage to spawning gravels and channel bottom biota.

Dam construction offers both hazards and benefits to the aquatic environment. During construction there is of necessity excessive local soil disturbance and considerable sediment production, but this can be reduced with foresight and care in the various phases of the job. Drainage from gravel-washing operations and from borrow pits can be diverted to safe disposal or ponded to trap sediments before release. Drainage from concrete mixing and curing may contain toxic substances and should be diverted away from the stream. Construction debris should be cleaned out of the stream below the project and from the flowage area above. Some Federal agencies now require clearing of trees and brush from reservoir flowage areas; this operation is generally desirable to protect water quality, to reduce floatable debris and flood hazards, and to safeguard recreation use, as well as to maintain the aquatic habitat. Once the dam is built and its reservoir filled, its operation can be managed to reduce flood peaks, to provide additional water to stabilize low flows, to provide water for filtration as may be needed downstream, and to modify water temperatures and oxygen content downstream.

Recreation areas are increasing in numbers and size and intensity of use everywhere, and present some special problems. Most recreation areas and use are associated with water; heavily used areas have compacted soil and often are readily eroded. Adequate drainage and drainage disposal may be necessary to avoid undue erosion and sediment production from streamside or lakeside recreation areas. Unless facilities for collection and pickup of litter and garbage are made available, these things may add to the waterborne debris load. Sewage disposal systems adequate to cope with weekend loads from use by thousands is also a real need at many favored recreation spots. Restrictions on motorboats on heavily used lakes may be necessary to keep oil and gas and fume pollution of lake water to a minimum to safeguard the aquatic environment.

REHABILITATION OF DAMAGED WATERSHEDS

Forest and range areas, denuded by fire, soil-disturbing logging methods, or overgrazing, may become badly eroded and incapable of revegetating naturally. These flood and sediment source areas need help; it can be provided by means of contour trenching to catch and infiltrate storm runoff; by gully stabilization with checkdams, bank-smoothing, and revegetation; by cultivation, reseeding, fertilization, and mulching on range lands; and by replanting forest trees. Major projects of such kinds are underway on public lands, but more rapid progress is needed to cover the great areas awaiting treatment.

Avalanche areas in the western mountains can be stabilized by rock and brush barriers and tree-planting. Work should concentrate first on the snow accumulation and breakoff points at the tops of the avalanche paths. Though avalanches are restricted to a relatively small area, they present serious problems locally and often carry huge loads of rock, soil, and organic debris to plug stream channels and cause floods.

On any cultivated land, erosion by wind or water may occur wherever the soil is left bare. Soil-conserving farming methods can prevent or greatly reduce erosion hazards. Where sheet and gully erosion are already well advanced, preventive methods may be useless; terracing, gully stabilization, conversion to forest, and other practices should be employed. These are well described in a number of State and Federal agricultural bulletins. We need only note here that it is quite possible to get immediate and large reductions in runoff and sediment movement from eroded areas by these methods. A planned program of watershed protection and development is needed on about a billion acres in more than 8,000 watersheds.*

Debris cleanup in streams and reservoirs may be necessary to restore the aquatic habitat to use and productivity. Old logging operations often put huge quantities of stumps, limbs, and cull logs into stream channels; usually these formed jams that blocked and diverted the stream, prevented fish passage, and increased sediment loads by bankcutting. Heavy loads of sediment deposited in the comparatively still waters behind a dam have filled or threatened to fill many reservoirs; as the waters become shallower they tend to become warmer, the advancing deposition deltas tend to obstruct fish passage, and reservoir bottoms become coated with the fine silt and clay particles that are the slowest to settle out. Sediment removal from the reservoir flowage area is sometimes possible; in other cases, sediment barriers can be installed upstream; but in any case, reduction of sediment yield by proper management and corrective action on the watershed lands will improve the situation.

Restoration of dredged areas along streams and in meadows, of quarries and borrow pits, and of strip-mine spoil piles is often necessary to open a stream course to unobstructed fish passage, to remove loose silt and sand and gravel from the reach of floodwaters; and to prevent contamination of streamflow by mineralized and silt-laden drainage. Often this may be best accomplished by stockpiling surface soil, filling and smoothing the mined area when the operation is finished, respreading the topsoil, and seeding and planting to re-establish plant cover, to provide shade, and to hold the loose material against erosion. A channel of sufficient capacity to carry normal high flows, with riffles, pools, and some cover for fish life, should be left open through the treated area. Some revetment or other bank stabilization measures may be needed until the re-established plant cover takes hold.

SUMMARY AND CONCLUSION

We have covered very rapidly and sketchily the natural, the developmental, and the use factors of the land that may adversely affect the aquatic environment. We have briefly noted their effects, and have touched upon various ways in which enlightened land management can protect and benefit the aquatic habitat.

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*C. J. Francis and R. G. Andrews, SCS, at National Water Resources Engineering Conference, quoted on page 213, June 1962 Water and Sewage Works Journal.

We have by no means covered all the possibilities. The intent has been to direct attention to and stimulate consideration of these problems on the land that are so intimately related to the problems in the streams.

Many things of significance have been omitted because they did not appear encompassed by the term "watershed management." One of these is the runoff from roofed and paved urban areas, runoff that carries a goodly load of dissolved and suspended materials unwanted in the aquatic habitat. Another is that of municipal and industrial wastes.

The emphasis has been on sediment production. In the forested mountains of the West, this appears to be the most important single factor. It is also important in the farming areas of the Middle West and the East. But in the areas with less forest and gentler topography and more farmland, it may well be that nutrients and toxins added to the streams from agricultural operations are much more significant.

Much of what has been mentioned in the way of preventive treatment or management might be accomplished by more effective control under existing State laws. Still there appears room for development of a new philosophy; i.e., that enterprises on the land should bear the same responsibility toward protecting water resources as that required of industries and municipalities that use the water.

DISCUSSION

Increased sedimentation of streams seems to be the most obvious effect of land use practices on the aquatic habitat. The addition of finer particles to the bottom gravels reduces the niches where many benthic organisms live. Perhaps the direct effects on eggs are among the most important. There is a smothering effect from silt coatings and a decreased permeability of the bottom gravels reducing the flow of water over the eggs. The turbidity resulting from the increase in suspended particles also reduces the light penetration and photosynthetic rate.

Not all sedimentation is a result of obvious operations such as mining and cultivation. Instances are on record in which the activity of ducks has reduced fish egg survival. Perhaps the trampling of stream banks by cattle may be far more important than is commonly recognized. Changes in the stream bank brought about by cattle or man-made channel changes may produce a cycle of changes, which may be carried clear to the mouth of the stream. Usually, these changes are not desirable.

The feeling among the discussants seemed to be that, ideally for fish production, partial tree cover of the banks, something less than complete bank stabilization, and an increase in rainfall infiltration of the soil are all desirable goals along with reduction in siltation and turbidity. However, the attainment of such goals may affect the stream flow patterns and reduce the total amount of water reaching the stream. In more arid areas, grasses are more desirable than trees because they achieve stabilization of soil but do not lose as much water through transpiration.

In any case, the optimum conditions for fish production will have to be sacrificed in many instances for multiple uses of the surface waters. However, the general feeling was that many improvements could be made that would improve the waters for fish production and still incorporate multiple use.

SOME ENVIRONMENTAL EFFECTS OF COAL AND GOLD MINING ON THE AQUATIC BIOTA

*A. D. Harrison**

INTRODUCTION

Gold and coal mining in South Africa, as in other parts of Africa, have marked effects on streams draining the mining areas. These effects are of two main types: 1. *Silting.* This is particularly marked in gold-mining regions where huge volumes of crushed rock are deposited in dumps as fine sand, and powdered ore is deposited in slimes dams after cyanide extraction. 2. *Pollution by acid sulphates.* During both types of mining, when pyrite is exposed to air and water, it is oxidized by bacteria into ferrous and ferric sulphate, with or without free sulphuric acid. In coal mining this takes place mainly in the mine; in gold mining most of the oxidation takes place in the outer layers of mine dumps and slimes dams. Water from mines and dumps drains into streams and, although free acid is not usually present, these effluents are usually highly acidic, the salts being hydrolyzed; pH values of 2.3 or lower have been recorded. Kemp, 1962, recorded acidities of 314, 588, and 750 ppm as $CaC0_3$ in Natal colliery effluents, and 10,700 ppm as $CaC0_3$ in a coal mine dump.

Silt from mining operations can have serious effects on streams and biota, but these are usually localized. Sandy silt soon settles and fine silt flocculates in the highly mineralized, acid water. Another form of silting arises from the oxidation of ferrous iron in the effluents to the ferric state. Insoluble ferric compounds are precipitated in a characteristic red or yellow deposit on the stream bed. The oxidation rate is greatly accelerated by iron-oxidizing bacteria, so deposition usually occurs along relatively short stretches of a stream. In the Witwatersrand gold-mining region this process normally is complete before the water reaches permanent streams. Where this is not the case, the biota is largely eliminated except for some larger plants such as *Typha latifolia*.

In South Africa pollution by acid sulphates is a major problem, especially as water remains highly mineralized after neutralization. The rate of acid neutralization depends on the surface geology; in some places streams run through dolomite or other carbonate-rich formations and the pH rises rapidly, but in many parts natural carbonates are not readily available and strongly acid conditions persist for many miles.

Research in South Africa has been concentrated on acid pollution and the subsequent contamination of water supplies with neutral sulphates.

EFFECTS OF ACID SULPHATE POLLUTION ON THE BIOTA

Polluted Streams With a pH Below 5.

Two streams with a pH below, or usually below, 5 have been investigated. The first was the Klip River, near Johannesburg, which is polluted by runoff from gold mines. It was studied not far from its source where, as a stream, it runs through a swamp called Olifantsvlei. Here the pH went above 5 only once during an 18-month study. Conditions are described in some detail by Harrison, Keller, and Dimovic, 1960. The other stream, the Klipspruit near Witbank, Transvaal, receives acid effluents from coal mines, and is reported on by Harrison, 1958. A fuller description is to be published by Mr. G. Venter of the Transvaal Provincial Administration.

Table 1 shows the chemical quality of these two streams as well as that of a mineralized but less acid stream. The pH of the water of the Klip in Olifantsvlei was usually between 4.2 and 5.0; only two values were recorded outside this range, 5.9 towards the end of a dry season, and 3.7 during the height of a wet season. The pH in the Klipspruit, Witbank, was 2.9 on two widely separated occasions; during Venter's study the pH went slightly higher than this.

The biological studies recorded here were carried out well below the main region of silt deposition and the formation of ferric compounds. Neither stream was subjected to scouring floods, so bottoms and banks were stable. The biota was similar to that found in normal dystrophic waters.

Macrovegetation. Table 2 gives the more obvious macrovegetation of the two streams, compared with that of the slightly acid to slightly alkaline Johannesburg Klipspruit, a tributary of the Klip River, which it joins in Olifantsvlei. The flora of this tributary was fairly typical of unpolluted and slightly polluted streams of the region. The most obvious vegetation change in the acid Klip was the absence of *Lagarosiphon major* and its replacement by *Scirpus fluitans*.

A characteristic feature of the two acid streams was the accumulation of undecayed and partially decayed vegetable matter in various parts of the stream bed. Most of Olifantsvlei was a muddy swamp, but the section through which the acid Klip flows has become a peat bog; in fact recent peat was being cut there. The fern, *Dryopteris thelypteris*, was a characteristic plant on the bog.

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Table 1. WATER CHEMISTRY OF STREAMS

	Johannesburg		Witbank
	Klipspruit	Klip	Klipspruit
pH Wet season	5.2 to 6.8	3.7 to 4.3	2.9
Dry season	6.1 to 7.8	4.0 to 5.9	2.9
Total dissolved solids, mg/liter	1,375 to 1,450	930 to 1,530	241 to 624
Sulphates, mg/liter	570 to 850	405 to 1,660 [a]	475
Chlorides, mg/liter	83 to 174	18 to 115	15
Calcium, as $CaCO_3$, mg/liter	140 to 650	290 to 650	
Magnesium, as $CaCO3$, mg/liter	40 to 260	125 to 300	
Turbidity, as SiO_2, mg/liter	trace or nil	nil	nil

[a] Total dissolved solids were not measured on this occasion.

Table 2. MACROVEGETATION OF STREAMS

	Johannesburg		Witbank
	Klipspruit	Klip	Klipspruit
Sphagnum truncatum	-	-	abundant
Dryopteris thelypteris	-	common	-
Typha latifolia	common	abundant	abundant
Paspalum sp.	common	-	-
Phragmites communis	abundant	common	abundant
Scirpus fluitans	-	abundant	-
Juncus exsertus	-	common	-
Juncus oxycarpus	-	common	-
Lagarosphon major	abundant	-	-
Polygonum lapathifolium	common	common	-
Ranunculus meyeri	common	common	-
Nasturtium officianle	abundant	-	-
Crassula natans	abundant	-	-
Alisma plantago	common	-	-
Cotula coronopifolia	common	-	-

The Witbank Klipspruit was very different in appearance from nearby, unpolluted streams. These latter were fast flowing and of the trout stream type. They had stony runs and stickles interspersed with small pools with sandy or slightly muddy bottoms. The banks were fringed with small marginal reeds and trailing grass; there was no true aquatic vegetation. The Klipspruit, on the other hand, was much overgrown by *Phragmites communis* and *Typha latifolia* and other reeds, and the bottom of its small valley was becoming swampy in places. Streamflow was impeded here and there by masses of partially decayed vegetable matter; this and stones were covered with a jelly-like growth of the diatom, *Frustulia rhomboides* var. *saxonia*. Deeper pools were floored with a thick growth of *Sphagnum truncatum*.

Strongly acid pollution does not eliminate macrovegetation and, although it discourages some species, it may actually encourage others; communities include some "acidophilic" species.

Algae. These were studied only in the Johannesburg streams. Algal growths were less dense in the acid Klip than in other nearby streams, but common filamentous types were often found, such as *Sphaerocystis* sp., *Oedogonium* sp., *Mougeotia* sp. and *Tribonema* sp.

The diatoms of the Klip were studied in some detail (Cholnoky, 1958, and his notes in Harrison, Keller, and Dimovic, 1960). Cholnoky found that the diatom community of the acid Klip consisted mainly of acidobiontic species, *Eunotia exigua*, *Frustulia magaliesmontana*, *Frustulia rhomboides* var. *saxonia*; and *Pinnularia acoricola*; the acidophilic species *Pinnularia subcapitata*; and the resistant species *Achnanthes microcephala* and *Achnanthes minutissima*.

The *fauna.* In Table 3, the fauna of the two Johannesburg streams is compared. That of the slightly acid to slightly alkaline Klipspruit was fairly typical of a slow-flowing stream in the district. The fauna of the acid Klip was impoverished, consisting of widespread species resistant to acid conditions. Faunal densities were somewhat lower than in normal streams, but some forms, such as the caddis *Oxyethira velocipes* and the oribatoid mite, *Hydrozetes* sp., were sometimes more abundant than in normal streams. This is not brought out by the percentage composition figures in the table. Note the lack of caenidae at both stations.

The fauna of the acid Witbank Klipspruit was greatly impoverished in species, although the faunal density was not particularly low. Normal streams nearby contained representatives of most of the usual aquatic groups and families such as Planarians, Ostracoda, Copepoda, river crabs, Hydrachnellae, Baetidae, Leptophlebiidae, Tricorythidae, Caenidae, Heptageniidae, several families of caddis, damselflies, dragonflies, the usual families of aquatic beetles, including Hydraenidae, Elmidae and Psephenidae, Simuliidae, Ceratopogonidae, and the common families of Chironomidae. The fauna of the acid stream lacked most of these and consisted mainly of *Hydrozetes* sp. and other oribatoid mites, the caddis *Oxyethira velocipes* and the chironomid *Polypedilum (Pentapedilum) anale.* Other species present were *Baetis harrisoni, Athripsodes harrisoni* (Leptoceridae) and the chironomids *Chironomus linearis, Endochironomus disparilis,* and *Tanytarsus pallidulus.* Venter will probably add to this list.

No fish were found in either of the acid streams.

The fauna of these strongly acid waters consisted mainly, if not entirely, of fairly widespread species that are resistant to acid conditions. Most of those recorded have also been found in distinctly alkaline waters. Some, however, appeared to be more abundant in these acid waters, which could be due to lack of competition.

Harrison and Agnew, 1962, discuss the fauna endemic to naturally acid streams flowing off quartzites in the Southern and Southwestern Cape Province, South Africa. There is a whole series of species, mainly Ephemeroptera, Plecoptera, and Trichoptera, as well as other groups, which are restricted to acid streams of the region with a pH fairly constantly below 6.0. In nearby streams from other geological formations, similar in all respects but with a pH above 6.0, these are rare or entirely absent and are replaced by other species that are widely distributed over Southern Africa. Some widespread species, however, are common or even abundant in the acid streams.

The acid stream region of the Cape Province is too far from the polluted Transvaal streams for the

Table 3. FAUNA OF AQUATIC VEGETATION IN JOHANNESBURG ACID AND SLIGHTLY ACID STREAMS

	Percentage composition	
	Klipspruit [a, e]	Klip[b]
Chaetgaster sp.	0.3 to 6.4	nil to 5.5
Nais sp.	nil to 1.4	nil to 13.0
Cyclops spp.	16.6 to 84.1	nil to 13.6
Paracyclops cf. poppei	nil to 1.5	p to 6.3
Austrocloeon virgiliae	p to 6.6	-
Enallagma glaucum	0.1 to 2.2	p to 1.5
Anax imperator	nil to 0.4	p to 0.1
Trithemis spp.	nil to 0.2	-
Orthetrum spp.	nil to 0.4	-
Athripsodes harrisoni	rare	occasional
Ecnomus sp.	rare	occasional
Oxyethira velocipes[c]	0.2 to 3.9	0.6 to 25.2
Pea pullula	nil to 1.7	-
Micronecta dimidiata	0.2 to 2.9	-
Dytsicid larvae	occasional	-
Culicini	occasional	occasional
Pentaneura spp.	nil to 8.3	-
Corynoneurinae	3.8 to 23.6	0.5 to 3.3
Tanytarsus-type larvae	nil to 1.9	occasional
Other Chironomidae	3.7 to 42.5	17.3 to 90.0[d]
Bezzia-type larvae	occasional	-
Hydrozetes sp. *(oribatoides)*	occasional	6.9 to 40.6

[a] pH 5.2 to 7.8; usually 6.5 to 7.0.

[b] pH 3.7 to 5.9; usually 4.2 to 4.8.

[c] Given as *Argyrobothrus* sp. in Harrison, 1958, and Harrison, Keller, and Dimovic, 1960.

[d] Mostly *Polypedilum (Pentapedilum) anale* Freeman.

[e] Entry p means present in very small numbers.

endemic species to have invaded them; however, some of the more widespread species found in the acid Cape streams are those that have been able to colonize the acid-polluted Transvaal streams. These include *Austrocloeon virgiliae*, *Baetis harrisoni*, *Enallagma glaucum*, *Athripsodes harrisoni*, and *Oxyethira velocipes*.

The chironomid that was most abundant in the acid-polluted streams, *Polypedilum (Pentapedilum) anale*, has a scattered distribution over most of sub-Saharan Africa. Where larval habitats have been identified, they have proved to be oligotrophic waters low in or lacking bicarbonates with an acid to neutral reaction, or distinctly dystrophic waters. This chironomid has been found in acid, polyhumic water at sea level near Cape Town (Harrison 1962), and in lakes just below the glaciers in the Ruwenzori Mountains, 12,500 to 13,000 feet, where the pH is about 4.5 (A.N. Adams, personal communication).

Acid-polluted streams with a pH constantly below 5.0 have a fauna impoverished in species but not necessarily of a low density. The faunal association is characteristic and consists of resistant or tolerant species and possibly a few that prefer acid conditions.

Slightly Acid or Fluctuating Conditions

Table 1 shows that pH conditions in the Johannesburg Klipspruit fluctuated somewhat, as acid from gold mines higher upstream were sometimes not completely neutralized. The pH seldom fell below 6.0, however, and the fauna and flora were more or less normal for the region, although the sampling station was near the end of a region of recovery from sewage works pollution. The diatom flora contained acidophilic elements but not acidobionts.

At a station below the junction of the acid Klip and Klipspruit, conditions fluctuated slowly during the survey period, and, after heavy rains during January and February 1955, the pH remained below 6.0 but above 5.0 for some months. This led to a dying off of thick growths of *Potomogeton pusillus* and a drop in faunal density. The species composition became somewhat impoverished, but the typical associations of a strongly acid stream did not develop. During the same period, thick growths of *Ulothrix* sp. appeared and died off periodically; however, not enough pH readings were taken to be able to correlate the death of the algae with pH figures. The fauna of stony runs, normally dense at this station, became very sparse. Caddis, such as *Cheumatopsyche afra* and *Macronema* sp., and *Simulium* spp. were present in very small numbers for a few months but finally disappeared. They returned after the pH had been above 6.5 for 2 or 3 months.

Oliff, 1963, describes conditions in Natal coal fields. Here again acid sulphates were produced, but were neutralized rapidly by carbonates in surface rocks and soils. There were no long, permanently acid stretches of stream below the deposition sites of silt and ferric compounds. Short stretches, where the pH was acid during the wet season and alkaline during the dry, had practically no fauna at all. Conditions here were probably made worse, however, by scouring floods.

Slightly acid conditions, i.e., pH values from 6.0 to 7.0, apparently have little effect on the biota except on the composition of some algae such as diatoms, even if the pH drops for short periods of a week or so into the 5.0 to 6.0 range. Prolonged drops into the 5.0 to 6.0 range produce most marked effects.

Effects after neutralization

After neutralization of acid sulphates, the streams in the mining regions carry large concentrations of the sulphates of calcium and magnesium. Sulphate values of over 1,000 milligrams per liter have been recorded. This type of pollution is very important since many, if not all, of the streams run directly into public water supplies. Large concentrations of neutral sulphates have no detectable effects on biological associations, however, at least in the concentrations recorded in South Africa.

The Johannesburg Klip River and similarly polluted streams find their way eventually into the Vaal River; this major river is the water supply of Johannesburg and many surrounding towns. Although most of the water of the Vaal comes from a higher catchment, such factors as a large storage dam higher up and irregular rainfall distribution result in disproportionately large contributions from mine-polluted tributaries at certain times. This raises the total dissolved-solids content appreciably. Normally the Vaal River water is turbid at all times of the year owing to extremely fine silt in suspension. This shows very little tendency to flocculate and it retards the growth of planktonic algae and the animals that feed on them. The effect of the mineral pollution is to bring about considerable flocculation. This allows unusual growth of plankton, which is most noticeable where the river is held back by a large barrage.

Oliff, 1963, dealing with Natal streams, reports increased algal growth below the neutralization zone. He considers this is due to nutrient salts coming from the coal mines. The increased algal growth leads to increased faunal density and certain changes in the species composition, rather like those found towards the end of a "beta mesosaprobic" zone.

Neutral sulphates, in the concentrations found in South African streams, i.e., not much higher than 1,000 milligrams per liter, have little noticeable effect on the biota. The only exceptions are where they result in abnormal clarity of water with increased algal growth and related changes. The growths may be assisted by nutrient salts from coal mines.

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THE EFFECTS OF STREAM SEDIMENTATION ON TROUT EMBRYO SURVIVAL

*John C. Peters**

INTRODUCTION

Small quantities of sediment are introduced constantly through the entire course of a stream by geological processes and have little effect on the aquatic communities occurring normally within the stream. The introduction of gross quantities of sediment into streams changes the quality and quantity of lotic aquatic life. Agricultural practices responsible for the addition of gross quantities of sediment into streams are: (1) Overgrazing range land, (2) brush and tree removal on the floodplain and along stream banks, (3) snag removal and channel realignment, (4) row crop production on steep sloping land, (5) overgrazing floodplain land, and (6) surface irrigation return water.

The addition of gross quantities of sediment changes the community of aquatic organisms within a stream and diminishes the total productivity of the stream (Cordone and Kelley, 1961). Trout or other sport fishes disappear and less desirable fish replace them in the stream community complex. Bottom fauna and fish production are reduced in streams carrying large sediment concentrations through the entire year.

Clean, permeable gravels provide the nursery areas in the stream environment for trout embryos. In the process of redd construction, sediments are cleared from the gravels by the spawning female before the eggs are deposited. The fertilized eggs develop in an environment well protected from predators. Water seeps through the gravels into the redd delivering oxygen to the developing trout embryos and washing away metabolic waste products produced by the embryos.

The suspended sediment present during the incubation period can greatly affect the survival rate of developing trout embryos. In a stream with large sediment concentrations, sediments will be deposited in trout redds, clogging the pore spaces between the gravels. Consequently, the seepage rate will decrease and the supply of oxygen to the redd will diminish.

Continuous large sediment concentrations in a stream during the trout egg incubation period can determine the recruitment of young-of-the-year trout to the population. Without high yearly recruitment, the trout population can be replaced by a species whose needs are better supplied by the environment.

This report of our investigation of Bluewater Creek, a stream greatly influenced by agricultural practices in its lower reaches, considers the relationships between stream discharge and sediment concentrations and their subsequent effects on the intragravel apparent velocity (seepage rate) and intragravel oxygen supply to the developing trout embryos.

METHODS

Sediment concentrations and discharge were measured at five sampling stations in Bluewater Creek, Montana, by standard methods used by the Geological Survey. The Department has a cooperative agreement with the Quality of Water Division of the U.S. Geological Survey in Worland, Wyoming, to collect these records. Methods used for sampling suspended sediment are found in reports by the Federal Inter-Agency River Basin Committee (1940, 1941). Stream-gauging procedure to measure discharge is summarized by Corbett (1943). Sediment concentration is defined as the weight of sediment in a water-sediment mixture to the total weight of the mixture and is ordinarily expressed in parts per million (ppm). Discharge is defined as the rate of flow at a given instant and is ordinarily expressed in cubic feet per second (cfs).

In the vicinity of the sediment-discharge sampling stations, man-made redds were constructed by excavating a hole in the streambed approximately 3 feet long, 2 feet wide, and 1 foot deep. The excavations were filled with 3/8-inch sorted gravel chips and allowed to stabilize for 1 week before eggs were placed in the redds.

Eyed rainbow trout eggs were counted, poured into Vibert boxes (Anon., 1959) partially filled with gravel chips, and placed in the redds. At each sampling station, two Vibert boxes with 200 eggs per box were placed 7 inches deep within the redd. The developing embryos were allowed to remain in the stream until 1 week after calculated hatching time.

Mortality is defined as the ratio of the number of dead embryos remaining in the Vibert boxes to the total number of eyed rainbow eggs placed in the egg-hatching box.

The Mark VI standpipe apparatus was used to measure the intragravel dissolved oxygen and intragravel apparent velocities within the redds containing the developing rainbow trout embryos. The theory and application of this standpipe are found in Terhune (1958). Intragravel dissolved oxygen determinations were measured with a microtechnique of the Winkler method (Harper, 1953).

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Description of the Study Area

Bluewater Creek is a spring-fed stream approximately 15 miles long, flowing in a northwesterly direction to its confluence with the Clarks Fork of the Yellowstone River near Fromberg, Montana. During the irrigation season, little water is diverted from the creek in the upper 6 miles. Irrigation demands, diverting water from the creek, and irrigation surface and subsurface returns, greatly change the quality of water in the lower 9 miles of the stream. During the winter months (November through March), fluctuations in flows are small.

Experimental Design

Sampling sites in the study stream were chosen so that comparisons could be made between stations with small sediment concentrations and stations affected by progressively larger sediment concentrations. The five sampling stations were located at intervals of about 3 miles and numbered consecutively I through V; with I denoted as the upstream station, V the downstream station.

FINDINGS

In the winter months, when no water is diverted from the stream for irrigation, the daily mean discharge at all five sampling stations showed little fluctuation (Figure 1). The greatest change between consecutive days in daily mean discharge was 2 cubic feet per second.

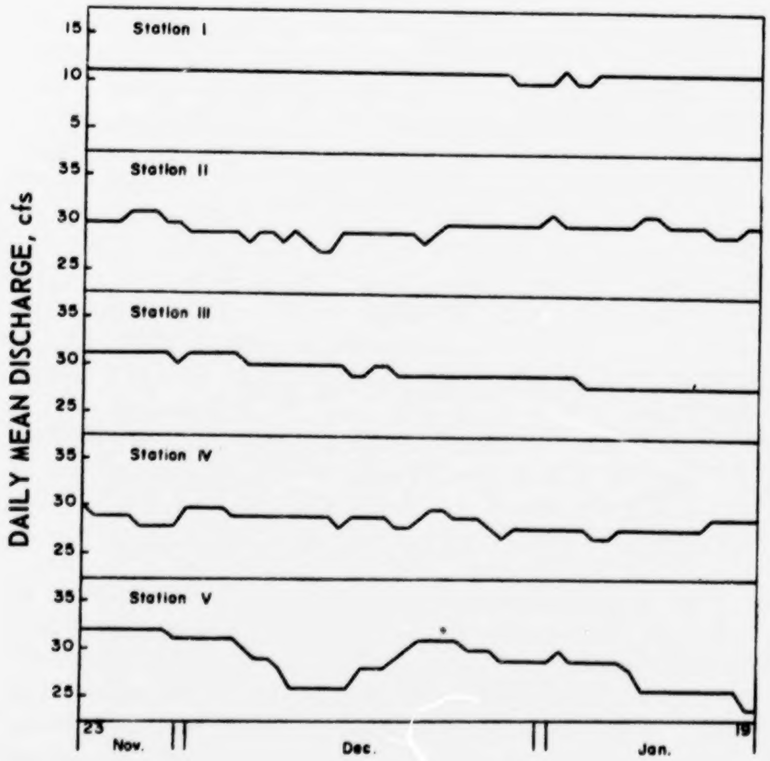

Figure 1. Daily mean discharge from five sampling stations in Bluewater Creek from November 23, 1961, to January 19, 1962.

The monthly average sediment concentrations for the five sampling stations are shown in Figure 2 and Table 1. The monthly average sediment concentration was smallest at the upstream station (I) of Bluewater Creek. Proceeding downstream, the monthly average sediment concentration increased progressively at the next two sampling stations (II and III). At sampling Station V near the creek's confluence with the Clarks Fork of the Yellowstone River, during November and December, the monthly average concentration was less than at Station IV.

In January, starting at the upper station and proceeding downstream to the lower sampling area, the monthly average sediment concentrations increased progressively.

Sediment concentrations at the upper station on Bluewater Creek were small, with 20 ppm as the largest monthly average concentration. This was roughly one-fifth of that at the next station downstream (II). A comparison of the monthly average sediment concentration at Station I with the other sampling stations shows that the concentrations were, at most, for Station III, 17 times greater; Station IV, 22 times greater; and Station V, 24 times greater. The sediment concentrations in Bluewater Creek can be characterized as increasing progressively downstream.

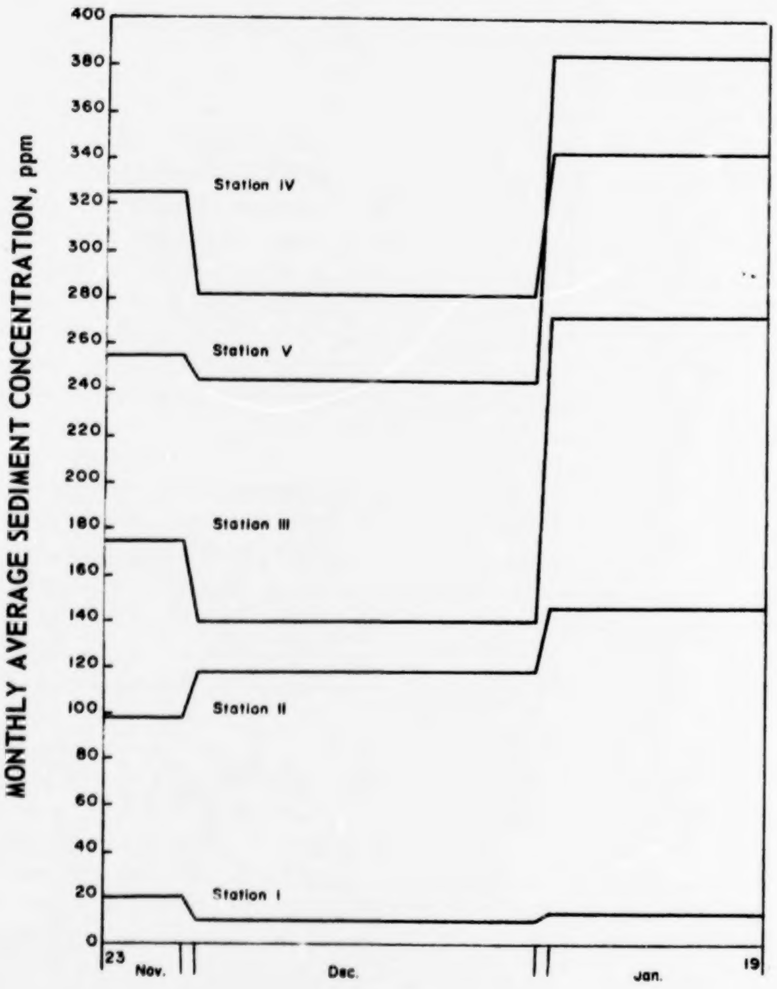

Figure 2. Monthly average sediment concentrations from five sampling stations in Bluewater Creek from November, 1961, through January, 1962.

Table 1. MONTHLY AVERAGE SEDIMENT CONCENTRATIONS AND RANGE OF OBSERVED CONCENTRATIONS IN BLUEWATER CREEK

Month		Sediment concentration, ppm Station				
		I	II	III	IV	V
November 1961	Monthly average	20	97	174	328	254
	Range of observed concentrations	9 to 67	61 to 166	115 to 389	249 to 481	196 to 427
December 1961	Monthly average	13	118	142	282	246
	Range of observed concentrations	8 to 26	59 to 203	69 to 199	55 to 884	79 to 511
January 1962	Monthly average	16	147	276	343	386
	Range of observed concentrations	12 to 25	71 to 363	172 to 486	221 to 506	61 to 1,240

The great variation occurring in daily mean sediment concentrations further describes the deterioration in water quality in the downstream areas in the creek. The sources of sediment with relatively stable flows are thought to be: Runoff occurring on warm days, lateral erosion of the streambanks, ice gouging of the streambanks and streambed, and degradation of the streambed. Little variation in daily mean sediment concentration implies that a stream in a steady state of operation would neither aggrade or degrade but transport the load supplied to it through the system without change in vertical position of the bed and without change in transverse form of the bed or the channel (Strahler, 1956). Bluewater Creek is relatively stable at the upper station (I) with sediment concentrations ranging from 9 ppm to 67 ppm in November. In January, the concentrations at the lower station (V), an unstable area of the stream, ranged from 61 ppm to 1,240 ppm. The progressive downstream deterioration in the creek, as indicated by great variation in daily sediment concentrations, points out relative stability in the upper areas compared with great instability in the lower areas.

The Mark VI standpipe apparatus was used to measure intragravel apparent velocity and intragravel oxygen concentration in redds placed in the vicinity of the sediment-sampling stations. A standpipe was driven into a redd constructed of 3/8-inch gravel chips and removed after each series of measurements.

The apparent velocity is laminar flow of water through the streambed gravel and is often called the seepage rate, the volume of liquid flowing per unit time through a unit area normal to the direction of flow. A high rate of seepage is required to deliver oxygen to the salmonid embryos and to carry away metabolic waste products (Coble, 1961).

In the stable upper area of the stream with small sediment concentrations, the apparent velocity through the man-made redds remained high during the entire study period (Figure 3). At the start of the study, the apparent velocities in each of the redds were within 5 centimeters per hour of each other. A marked progressive rate of decrease in apparent velocity was measured at each successive sampling site. After 12 days at the lower sampling station, the apparent velocity dropped from 85 to 25 centimeters per hour. No change occurred in the apparent velocity at Station I during the same time period.

Large concentrations of intragravel dissolved oxygen are required for salmonid embryo survival (Wickett, 1954 and Coble, 1961). At the start of the study, the dissolved-oxygen concentration in four of the five redds was 8.1 ppm; in the remaining redd at Station V, the dissolved-oxygen concentration was 7.9 ppm. The rate of decrease in dissolved-oxygen concentrations is shown in Figure 4, which illustrates progressive downstream deterioration in the redds. The least dissolved-oxygen deterioration occurred at Station I with a decrease of 0.7 ppm. At the lower two sampling stations the dissolved-oxygen deterioration was the greatest with a 1.7 ppm decrease at Station IV and 1.5 ppm decrease at Station V.

The average dissolved-oxygen concentrations also describe the progressive downstream deterioration in the creek (Table 2). The upper areas of the stream with relatively small sediment concentrations and high intragravel apparent velocities have large average intragravel dissolved-oxygen concentrations. Large average intragravel dissolved-oxygen concentrations are found in the downstream redds affected by high sedimentation rates and low intragravel apparent velocities.

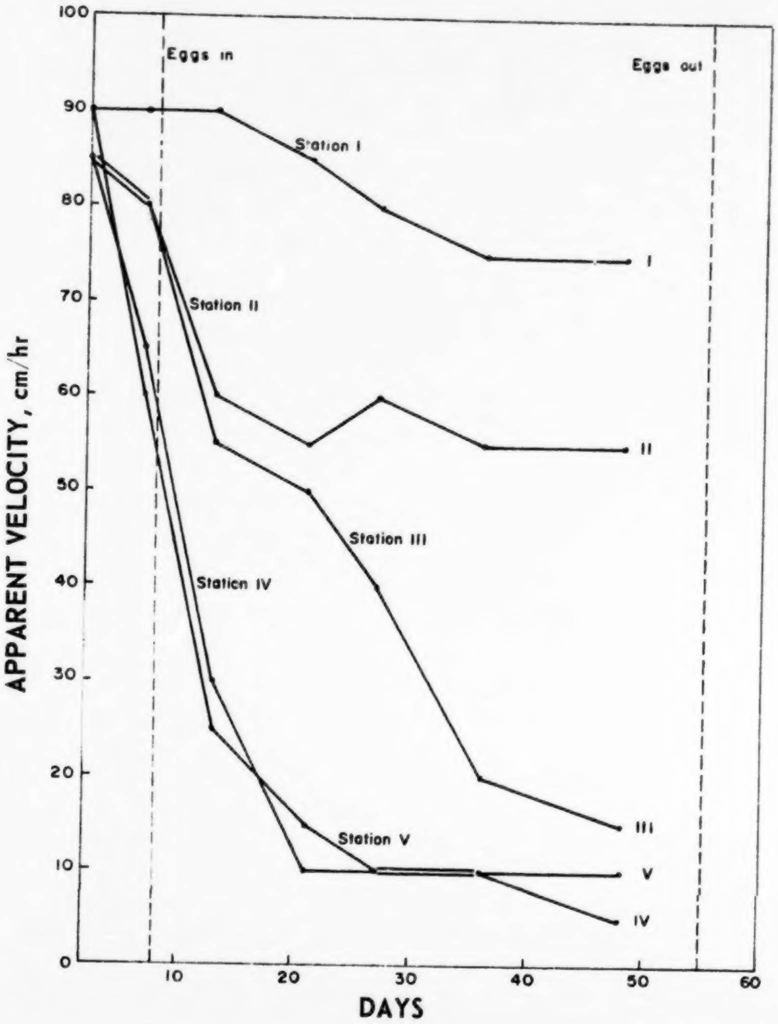

Figure 3. Intragravel apparent velocity at five sampling stations in Bluewater Creek from November 23, 1961, to January 8, 1962.

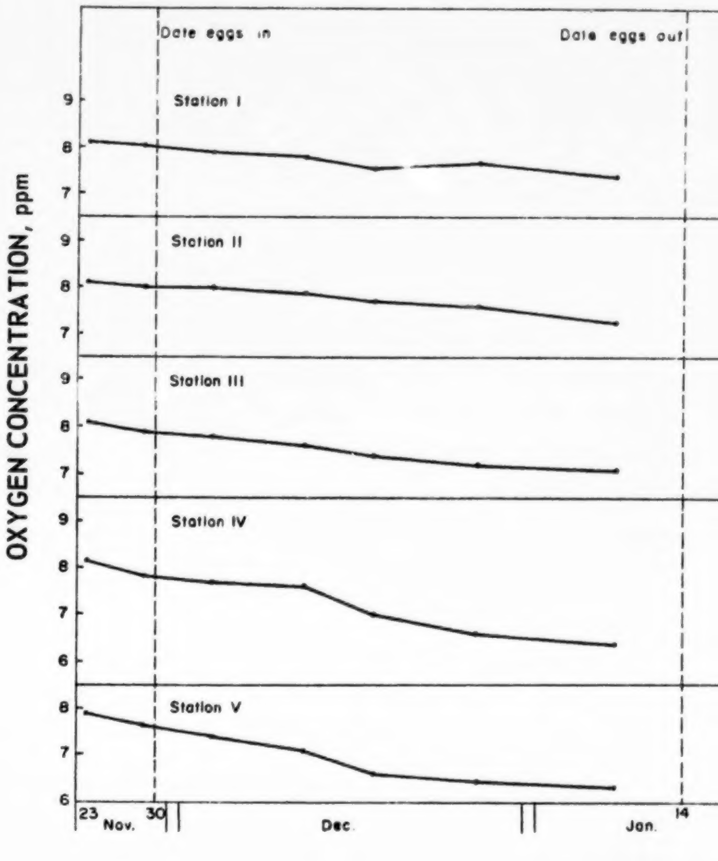

Figure 4. Intragravel oxygen concentration at five samplin stations in Bluewater Creek from November 23 1961, to January 8, 1962.

Table 2. TROUT EGG MORTALITY COMPARED WITH INTRAGRAVEL OXYGEN CONCENTRATIONS AND INTRAGRAVEL APPARENT VELOCITIES

Station Number	Mortality, %	Oxygen concentration, ppm		Apparent velocity, cm/hr	
		Average	Range	Average	Range
I	5	7.8	7.4 to 8.1	82	75 to 9
II	39	7.8	7.3 to 8.1	61	55 to 8
III	90	7.6	7.1 to 8.1	43	15 to 8
IV	100	7.3	6.4 to 8.1	21	5 to 9
V	100	7.1	6.4 to 7.9	23	10 to 8

The mortality of rainbow trout embryos is related to the average intragravel oxygen concentration and the average apparent velocity (Table 2). Only 5 percent of the rainbow trout embryos failed to survive at Station I. Mortality increased progressively at the next two downstream sampling stations. No embryo survival was found at the two lower stations in the stream.

DISCUSSION

Bluewater Creek, during the study period, v characterized as a stream with little fluctuation discharge. There was a progressive downstre increase in sediment concentrations at the f sampling areas in the stream. Man-made redds fil with 3/8-inch gravel chips were placed in the vicir of each sediment-sampling station. Each redd, at the start of the study, had almost identically large intragravel dissolved-oxygen concentrations and intragravel apparent velocities.The intragravel dissolved-oxygen concentration rate and apparent velocity decreased progressively downstream in relation to the progressive downstream increase in sediment concentration. Accompanying the progressive downstream decrease in intragravel dissolved-oxygen concentrations and intragravel apparent velocities was a progressive increase in trout embryo mortality.

Sediment passing a given area of a stream can greatly affect trout embryo survival. Small sediment concentrations with small fluctuations in discharge in a stable streambed environment indicate a stream area with a potential for good trout embryo survival.

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THE CONTROL OF FISH DISEASES AND PARASITES

*S. F. Snieszko, Chairman**

THE CONTROL OF BACTERIAL AND VIRUS DISEASES OF FISHES

S. F. Snieszko

Infectious diseases of lower and higher vertebrates are similar in many respects and are caused by the same types of agents, such as viruses, bacteria, fungi, and protozoan and metazoan parasites. For this reason control of communicable fish diseases is based on the same general principles as in all vertebrates.

There are, however, two very important differences in the approach to the study of fish diseases. One is that fish live in water and breath oxygen dissolved in water, instead of oxygen from the air. There is about 21 percent oxygen in the air, but the concentration of oxygen dissolved in water varies greatly. Sometimes fishes are exposed to water supersaturated with oxygen; this may result in fatal gas embolisms. At other times the lack of oxygen causes asphyxiation. Often the same body of water may be supersaturated with oxygen during the day and almost free from it at night. Since fish are poikilothermal, temperature is not only a limiting factor in fish survival, but variation in temperature affects the rate of fish metabolism, growth, production of antibodies, and the degree of resistance to pathogens and parasites. Considerable and frequent variation in the physical and chemical environment in which fish live also has a very far reaching and still inadequately appreciated influence on the incidence of diseases and effectiveness of control measures, as well as on the degree of side effects of drugs and disinfectants. There are also species-specific differences in the sensitivity of fish to various chemical agents. All this must be kept in mind when discussing control measures for communicable fish diseases.

In conducting bioassays with fish, there are frequent difficulties caused by columnaris disease and protozoan and metazoan parasites. Fish used for assays are often infected or parasitized, and the methods of control are still inadequate. Even though some of these infections are apparently not very harmful, they do introduce possible variables.

Starting with virus diseases, I should like to talk first about lymphocystis and fish pox of warmwater and aquarium fishes. Both diseases can be easily diagnosed by microscopic examination. Both produce hyperplasia of epithelial cells, are seldom fatal, and under natural conditions occur during the summer. Most of the fish recover by fall.

Salmonid fishes are often subject to acute systemic virus diseases that cause heavy mortalities in young fish. The most important are infectious pancreatic necrosis, virus diseases of chinook and sockeye salmon, and liver-kidney syndrome of rainbow trout. Etiology of the last disease is still disputed. There is no treatment known for virus diseases of fishes. The only control measures are sanitation, isolation, and selection of healthy brood fish.

Bacterial diseases of fishes are very common. Probably the most frequent are infections caused by *Aeromonas liquefaciens* (many synonyms) and *Pseudomonas fluorescens.* These bacteria may cause local lesions, necrosis, and systemic infections. Mortality rate is high, and fish may have latent infections that flare up when the fish are exposed to stress. Diagnosis is possible only by bacteriological identification of the pathogens.

A. liquefaciens and *P. fluorescens* are ubiquitous water bacteria but only cause diseases and losses among fishes with lowered resistance. German and Russian investigators suspect viral infections to be the most important predisposing factors. Other investigators attach more significance to environmental factors, as well as to bruises and stress caused by rough handling. Chemotherapy is usually effective in control of these infections. Chloramphenicol, tetracycline, and oxytetracycline given orally at a rate of 50 to 100 mg/kg/day are very effective. Nitrofurans are very promising. Sulfapyrimidines given orally at a rate of 100 to 200 mg/kg/day usually are also beneficial. Unfortunately, reinfections occur readily unless stress conditions are corrected. Very effective prophylaxis lasting for 1 to 2 weeks can be achieved by a single intraperitoneal injection of chloramphenicol, tetracyclines, and streptomycin at a rate of 10 to 20 mg/kg. Chloramphenicol succinate, which is readily soluble, seems to be toxic to fishes when injected intraperitoneally. Sanitary measures, uncrowded conditions, clean water, sufficient oxygen, and optimal temperatures are the best preventive measures.

Furunculosis is a systemic bacterial disease that most frequently affects salmonids. From salmonids it may be transmitted to warmwater fishes and cause rapidly progressing epizootics with a very high, often complete, loss of infected fish. It is caused by *A. salmonicida.* Positive diagnosis is possible only by identification of the pathogen.

Treatment and prophylactic measures are the same as for infections caused by *A. liquefaciens* and *P. fluorescens.* One of the best long-range preventive measures against the above described diseases is selecting and breeding resistant fish.

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Very important and common bacterial fish diseases are caused by myxobacteria. Gill disease is the most common in salmonid fishes. It is characterized by superficial infection of gills resulting in hyperplasia of gill epithelium, fusing and clubbing of gills. This results in distress caused by respiratory difficulties. If not checked in time, gill disease usually results in gill necrosis and death. This seems to be a typical stress disease because its occurrence is associated with crowding and accumulating nitrogenous waste products. The best prophylactic measure is clean, well-oxygenated water of optimal temperature. Treatment and prophylaxis can be accomplished by 30 to 60 minute exposure to quaternary ammonium disinfectants as Roccal or Zephiran, or to organic mercurials as pyridylmercuric acetate of ethylmercuryphosphate (Lignasan X). All these disinfectants are used in concentrations of 2 ppm calculated on the basis of active undiluted compound. Lignasan X, containing only about 6 percent active ingredients, however, is used only at a rate of 1 to 2 ppm of the product as is, and not on the basis of the active ingredient. Mercurials are very toxic to rainbow trout.

Columnaris disease is the other common myxobacterial disease of cold and warmwater fishes. It can produce different types of external lesions, ulcers, necrosis, and systemic infections. (Crowding, pollution, and increasing water temperature seem to be the most important stress factors.) The causative bacteria can be easily identified by their morphology and characteristic oscillating and sinuous motility. Antibiotics such as chlortetracycline and oxytetracycline are very satisfactory for control of external columnaris disease in aquarium fish when added to water at a rate of 20 to 60 ppm. In systemic infections sulfonamides are helpful. Recently completed *in vitro* studies indicated that nitrofurans and other antibiotics may be of use in the treatment and control of this disease.

Kidney disease and mycobacterioses are also important bacterial diseases. Kidney disease is common among salmonid fishes and perhaps some tropical species. The etiological agent is an incompletely described but very fastidious corynebacterium. Diagnosis is made on the basis of gram positive diplobacilli in smears prepared from the lesions. Erythromycin given orally at a rate of 100 mg/kg/day is the most effective drug.

Mycobacterioses are common among aquarium and marine species of fish. They usually cause chronic, systemic infections with multiple foci in visceral organs. In advanced cases mycobacteria are extremely abundant in tissues, and their presence can easily be demonstrated in acid-fast stained smears. The infected fish are stunted and often unable to reproduce, and the disease ends in death. Therapeutic measures are still unknown. Transmission occurs, most likely, from fish to fish and can easily be accomplished by feeding tissues of infected fish.

This brief survey has shown that there are numerous bacterial and viral fish diseases. Some can produce rapidly spreading epizootics with high, sometimes total, losses. Diagnosis is usually easily accomplished by isolation and identification of the pathogens. Some external and internal therapeutic measures are effective, and prophylaxis with drugs is often practicable.

The most important control measures are sanitation and removal of stress conditions. A very effective approach to the control of some infectious fish diseases is selecting and breeding resistant fish. This has been done successfully in a number of cases in Europe and the United States. The brood fish selected had survived without apparent harm a natural or artificial epizootic. In the next generation fish used for breeding were challenged by inoculation. Again the fish that were selected had survived without disease symptoms or were only lightly diseased. These were used as brood stock. By such a simple procedure it was possible to select and breed trout resistant to furunculosis and ulcer disease and carp resistant to hemorrhagic septicemia. All three are common bacterial fish diseases.

Fish are also subject to neoplastic diseases. Almost any type of tumor found in higher vertebrates has a counterpart in fishes. Recently hepatoma in rainbow trout reached pandemic proportions. In some hatcheries and in some lots of rainbow trout the incidence of hepatoma was close to 100 percent. In brook trout raised in hatcheries there is a common visceral granuloma of unknown etiology. It causes heavy losses among 2- and 3-year-old trout.

One must remember that, so far, there are no sources for obtaining fishes reasonably free from communicable diseases. Fish that have viral and/or bacterial or infectious diseases, latent infestations, and neoplastic or nutritional diseases and that are genetically heterogenous are hardly suitable for communicable diseases research, toxic chemical assays, or other investigations.

In contrast there are commercial and other sources for obtaining healthy and genetically uniform white mice, rats, rabbits, guinea pigs, and other animals. With the rapidly increasing interest in fishes as experimental animals, it would be most desirable if a fish breeding institution could be established from which to obtain healthy experimental fish. Such fish should be reasonably free from bacterial, viral, and parasitic diseases. They should be maintained under well-standardized conditions and fed diets to prevent malnutrition. Perhaps with the cooperation of federal, state, university, and commercial laboratories that use fish for experiments and bioassays, such fish hatcheries could be established and maintained. I would like to suggest the organization of a committee to investigate the desirability and other aspects of creation and operation of such an institution.

THE CONTROL OF FISH PARASITES

*Glenn L. Hoffman**

As stated in the preceding paper, healthy fish are desired for experimental work in bio-assays of pollution, in fish physiology and nutrition, chemical fish control, insecticide assays, and fish diseases. Parasitic infection is one of the many variables to be avoided in such experiments.

The parasites of fish belong to several taxons but, for convenience, we often categorize them according to their location on or in the fish. In his 1953 text, Dr. H. S. Davis divides the parasites into *external* and *internal* parasites. This terminology is particularly useful in the case of the treatment of ectoparasitic diseases because certain methods are effective for several groups of external parasites.

The life cycles of the parasite should be kept in mind as this may influence the type or time of attempted parasite control. In general, the fish parasites follow these patterns:

1. Ectoparasitic protozoa - most of these divide by binary fission, a few by internal budding *(Trichophrya)* and one by division within a cyst *(Ichthyophthirius)*. Typical protozoan cysts are not formed or are unknown for others. Infection is direct, from fish to fish or by contaminated equipment. The amount of damage to skin and gills is usually related to the number of parasites present.

2. Internal protozoa — some live in the intestine and divide by binary fission and also produce cysts. Infection is probably accomplished by cysts. Others live in tissues and divide and produce a mass of spores that is usually visible to the naked eye. Fish may be infected by ingesting spores that are freed from other fish. The protozoa that live in the blood stream divide by binary fission and are transferred from fish to fish by leeches in which they multiply. Damage to the fish ranges from negligible to fatal.

3. Monogenetic trematodes — except for *Gyrodactylus*, which is viviparous, these lay eggs that soon hatch; fish infection is from these larvae or adults from adjacent infected fish or contaminated equipment. Heavy infections may overwhelm small fish.

4. Digenetic trematodes — all of these have a snail intermediate host; fish may become infected by larval stages that escape from the snail or from eating other infected intermediate hosts such as larval insects, small fish, etc. Most of these are not serious pathogens, but large numbers of cercariae may kill fish. The eggs of blood flukes may plug so many gill blood vessels that the fish may be killed.

5. Cestodes — intermediate hosts are invertebrates (usually copepods) and sometimes small fish also. Rarely do these kill fish, but the larval stages may cause sexual castration and, in one instance, we have studied mortalities caused by larvae in the heart.

6. Acanthocephala — these are similar to cestodes.

7. Leeches — these are oviparous, and may live off the fish for long periods. Heavy infestations will kill fish and they are the vectors of fish trypanosomes, *Crypotobia*, and perhaps others.

Parasitic copepods — Their visible egg sacs are borne by females on gills, fins, or body of fish. Eggs drop off, hatch, go through several larval stages, attach to gills of fish, mate, and females move to the final site of attachment. The adults may produce ugly and sometimes fatal lesions. Massive infestation with the larvae on the gills may suffocate the fish.

PROPHYLAXIS

When fish are obtained for experiments, it is not wise to assume that they are not infected with parasites, particularly ectoparasites. Therefore, it is judicious to handle them as if they were infected. The best method is to examine them and to treat infected fish with formalin or potassium permanganate before putting them in clean facilities. They should then be kept in "quarantine" for 2 to 8 weeks. Most ectoparasites will have been eliminated by the chemical treatment, but *Ichthyophthirius*, and perhaps others, may remain alive under the epithelium of the fish; if so they will probably become evident during the "quarantine" period. In some cases it may not be feasible to treat the fish; in this case "quarantine" is even more important. During this period serious infection with internal parasites might also become evident. If certified parasite-free fish stocks could be obtained, as Dr Snieszko has indicated, this would be much better than using the above method. In the future it is hoped that lab-reared, parasite-free fish can be had.

METHODS OF CHEMICAL TREATMENT

Dipping. The fish are immersed in a dip net in the disinfectant for the required length of time. This method is useful when infected fish are transferred to clean facilities, or to reduce the parasite population on the fish.

Prolonged treatment. The required amount of chemical is added directly to the aquarium, pond, etc. and allowed to remain for the prescribed length of time. It is then drained and fresh water is added.

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Indefinite prolonged treatment. As above, but the pond, etc., is not drained; the disinfectant is allowed to dissipate.

Concentrations and dosages. Hardness, temperature, pH, organic matter, and probably other factors influence disinfectants; therefore, each installation should test the suggested concentration on a few fish before treating the entire stock.

TREATMENT AND PREVENTION

1. Fungi
   Ichthyosporidium - avoid feeding raw sea fish.
   Dermocystidium - treatment unknown.
   Saprolegnia and related fungi - malachite green, 1:15,000 dip for 10 to 30 seconds; 0.3 ppm for prolonged treatment of 1 hour; 0.1 ppm for indefinite prolonged treatment.
2. Protozoa
   External protozoa - treatment listed in order of choice: (1) formalin, 1:4000 to 1:5000 for 1 hour at 32° to 60°F; 1:6000 for 1 hour at 60° to 85°F; 15 to 25 ppm for indefinite prolonged treatment; (2) potassium permanganate, 1:1000 for 30 to 40 seconds; 10 ppm for 5 to 30 minutes; 2 ppm indefinite prolonged treatment in ponds, but 1 ppm in relatively clean aquaria; (3) pyridylmercuric acetate (PMA), 2 ppm for 1 hour (toxic to rainbow and cutthroat trout); (4) acetic acid, 1:500 for 1 to 2 minutes; (5) sodium chloride, 1 to 3 percent for several minutes to 1 hour.

One treatment usually suffices to break an epizootic except for *Ichthyophthirius* disease, which ordinarily must be treated on alternate days until there is no longer any sign of "Ich". This may take 5 weeks at 40° to 54°F, and 11 to 13 days at 60° to 70°F. Daily changing of the water or a rapid continuous flow will reduce or eliminate many ectoparasites including "Ich".

Most ectoparasites have optimum temperatures, which are similar to those of the host, but there are at least two genera of parasites that may be controlled by changing the temperature of the aquarium. The optimum temperature for *Chilondonella* is 40° to 50°F. This parasite is usually of no consequence in environments of 60°F or higher. The optimum for "Ich" is about 60° to 75°F; there is seldom trouble from "Ich" at 50°F or lower, and 90°F will kill the tomites. The latter temperature can be used to control "Ich" on tropical fish.

Intestinal protozoa

Hexamita — may be controlled with carbarsone or calomel mixed with food at 0.2 percent for 5 days. Yasutake et al (1961) found 2-amino-5 nitrothiaxole and p. carbamidophenyl arsenoxide to be more effective. Uzmann(1961) found one "strain" of *Hexamita* to be nonpathogenic.

3. Trematoda

Monogenea *(Gyrodactylus, Dactylogyrus,* etc.) — use methods for external protozoa listed above.

Digenea (intestinal) — di-n-butyl tin oxide has been used at 0.3 percent of the weight of food (Allison, 1957).

Digenea (metacercariae and adults in tissues) — no treatment known. Cercariae can kill fish if numerous. Control of snail host is sometimes possible. $CuSO_4$ at approximately 3 pounds per 1000 sq ft (cf. Mackenthun, 1958, for concentrations,etc.), and sodium pentachlorphenate at 5 ppm have been used to control snails; the latter will kill fish, but the chemical dissipates in 3 days. Control of the bird or mammal final host is sometimes possible.

Molluscicides are being intensively investigated by schistosome workers.

4. Cestodes

Intestinal forms — di-n-butyl tin oxide added at 0.3 percent of the food weight for 1 day will remove cestodes. Kamala at 1.5 to 2 percent of the food for 1 to 2 weeks, or by oral capsule at 0.2 gram per kilogram per day has also been used. There is no known control of the intermediate hosts of cestodes.

Plerocercoids in viscera and muscle — no known treatment. Some success has been achieved in controlling *Triaenophorus* by reducing the population of the final host, *Esox lucius.*

5. Nematoda

Intestinal forms have been removed from sturgeon with Santonin at 40 milligrams per fish.

6. Acanthocephala

There is no known treatment for acanthocephala of fish. Possibly the isolation of fish and control of intermediate hosts would be helpful.

7. Hirudinea

Leeches may be removed with the wettable powder of the gamma isomer of benzene hexachloride (lindane, Gammexane) at 0.1 to 0.5 ppm. The solvent of the nonwettable type may be toxic to fish; use cautiously. Sodium chloride at 1 to 3 percent for up to 1 hour may kill some leeches. Schaperclaus (1954) recommends quick lime at 0.5 gram per liter for 5 seconds.

8. Parasitic copepods

Benzene hexachloride and salt may be used as above. Ethyl parathion at 0.06 to 0.13 ppm has been used by Osborn (1961). Potassium permanganate at 2 ppm for indefinite, prolonged treatment kill some. Adult *Lernaea* are difficult to kill, but the larval stages are vulnerable to formalin, etc.

9. Beetle larvae predators

Kerosene or vegetable oil at 1.5 gallon per acre are recommended.

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DISCUSSION

Chairmen: Dr. S.F. Snieszko and Dr. Glenn L. Hoffman, Bureau of Sport
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The first topic of discussion was the control of the copepod parasite, *Lernaea.* The question was asked whether *Lernaea* could be controlled in a 2-acre lake. Dr. Hoffman doubted that it could be done. However, Bender (U.S.P.H.S., Cincinnati) stated the control of the parasite has been accomplished in golden shiners using benzene hexachloride at 0.1 ppm in treatments spaced 3 or 4 weeks apart. It was mentioned that Schaperclaus in his 1954 book entitled, "Fischkrankheiten," described the use of DDT at 1 to 50,000,000 for their control. It was stated that formalin had been used to kill larval copepods. The possible use of parathion and malathion was mentioned.

The subject of snail control was introduced. Snails constitute the intermediate hosts of many fish parasites. The rapid flooding of an area with rainwater containing 0 to 6-7 ppm of dissolved oxygen was mentioned. The use of shellcracker sunfish as a means of controlling snails was described. Experiments are under way in Puerto Rico using shellcracker sunfish to control snails of the genus *Austrolorbis* that carry the intermediate stages of human blood flukes. Shellcrackers were described as being voracious snail eaters.

There followed a discussion of the holding of marine fish in ponds of a size up to 150 m in length. The case of whirling disease in mullet was described. Whirling disease in trout and pond fishes has been attributed to dietary deficiency and eye flukes and to myxosporidian infection of the auditory capsule.

In the discussion of columnaris disease, the question arose whether anyone had ever controlled it in a pond or larger body of water. Dr. Snieszko reported an instance at Leetown in which 1 ppm of copper sulphate was added to a smallmouth bass nursery pond in which the disease was arrested. Surber had made this treatment and he reported another instance in which a 0.5 ppm treatment of copper sulphate arrested Columnar is in a pond near Cincinnati, where adult bluegills were being held in liveboxes.

The subject of leech control was brought up. Dr Hoffman stated that leeches had been controlled with benzene hexachloride but he did not know how much had been used. The control of tadpoles in fish ponds was discussed but no one knew of any method that would control them and at the same time permit survival of the fish. Dr. Snieszko reported that columnaris disease had been found in tadpoles and that their presence in the wild might have led to some of the heavy fish kills now being reported. He told of the presence of columnaris in coldwater fishes in streams of the west coast where the disease had been reported in water with a temperature of 10°C or less. Sulfonamides and terramycin could be used for the control of the disease. The occurrence of columnaris disease in the Columbia River was partly attributed to a series of impoundments created on the Columbia River that increased water temperatures. Increases in the acidity of the water also decreases the resistance of the migrating fishes. Howard Zeller of Georgia, reported a serious columnaris outbreak among golden shiners and bass in a 110-acre Georgia lake. Surber reported a selective kill of white crappies by columnaris in two farm ponds, one above the other, near Leesburg, Virginia. Both of these ponds contained good populations of bluegill sunfish, pumpkinseed sunfish, and largemouth bass that were unaffected by the disease that wiped out the white crappies. Mulligan reported instances in which shad and carp died from columnaris disease where bass and crappies were not affected. Stresses such as overpopulation, handling, and excessive temperature are believed to be factors in the outbreak of the disease.

Thomas (Alberta) described the increase in the number of Japanese snails in Lake Erie and inquired whether it was an intermediate host for any fish parasites. These snails are being killed by acrolein used for the control of algae and weeds along the Lake Erie shore. He described how fish were driven away by the presence of the herbicides. Mr. Fisher (Missouri) inquired whether carp become heavily parasitized. Dr. Hoffman replied that they were sometimes known in Europe to be quite heavily parasitized with monogenetic trematodes.

Thomas reported that *Triaenophorus* (tapeworm) infestation of tullibees could be controlled in part by manipulation of the pike population that harbors the adult worms. Another parasite that causes fish kills in tullibee populations is an ergasilid parasite *Lernaea versicolor.* It is reported that this species had a seasonal reproduction cycle that lasted about 2 weeks in summer.

Dr. Snieszko described how it had taken 3 years to rear furunculosis-free rainbow trout at Leetown, W. Va. There, he stated, they have kept the uninfected fish side by side with infected fish by strict prophylactic measures and by using different utensils for each tank. Dr. Kranz of Pennsylvania, described immunological techniques used to inject *Aeromonas* in fish. There they found that trout could be immunized in the same way that humans are immunized by injecting mineral oil mixtures of attenuated bacteria numbering 4,000,000 to 5,000,000 cells per cubic Milliliter. He reported the immunization of trout only 2 inches long.

Dr. Snieszko called upon his long time associate and friend, visiting Cincinnati from Krabkow, Poland, to describe their methods of selecting fish for maximum growth and a higher level of health. Dr. Zarnecki described their fish-cultural procedures with carp in which they graded young carp after they had been in ponds for 4 weeks. The young carp were graded into three groups and the medium and large-size fishes were marked by removing pelvic fins and placed in ponds where they grew much more rapidly than the smaller-size fish. The selection produced a 14 to 18 percent increased growth rate as well as healthier fish.

Dr. Snieszko reported that Dr. Schaperclaus obtained furunculosis-resistant trout by exposing trout to the disease while they were still very young.

Hepatoma disease was reported to affect most of those fish growing at a very rapid rate and eating voraciously. In this disease, cancerous growths develop in the liver of fish as a result of eating certain components of a dry meal diet. A cooperative effort is now being made to determine which of the dry meal diet components is responsible.

A discussion of prophylactic measures against furunculosis then followed. Mr. Papier of Ohio, asked whether PMA deteriorated with time. The answer to this was "no". Furacin is now being used as a prophylactic agent for the control of furunculosis. It is very stable, but when added to liver, breaks down in a few hours. Mr. Papier inquired whether any antibiotics had been used in ponds for parasite or disease control. The answer to this was in the negative.

Durrel, as long ago as 1928, found a bacteriophage for *Aeromonas salmonicida* to be more prevalent in ponds where furunculosis occurred. Joe Hahn, Michigan State, stated that no spore formers had yet been described among the bacterial diseases of fish.

Dr. William Beckman of the World Food Organization, stated that a world list of specialists in fish diseases has 3,500 names.

Dr. Snieszko reported the availability of a series of fish disease leaflets put out by the Bureau of Sport Fisheries and Wildlife. Each leaflet treats a separate disease and contains the basic information on the disease and its control. These can be obtained by writing to the Department of the Interior, Bureau of Sport Fisheries and Wildlife, Division of Information.

Inquiry was made regarding availability of textbooks on fish anatomy. The inquirer was referred to Schaperclaus' "Fischkrankheiten," which was published in 1954 but is now out of print, and to Ambecher's Teichenbuch der Fischkrankheiten, 1962.

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DETERMINATION OF THE CAUSE OF FISH KILLS

*G. E. Burdick, Chairman**

SOME PROBLEMS IN THE DETERMINATION OF THE CAUSE OF FISH KILLS†

G. E. Burdick

In the past 20 years the relationship of other chemicals and conditions to the toxicity of pure compounds has been studied rather intensively. These contributions are important but not too helpful in finding who, or what, killed the fish. In fact, it is almost impossible to establish the cause of a fish kill if a positive identification and determination of the conditions, chemicals, or complexes contributing to death are required.

Suppose a small, nontoxic quantity of copper is added to water. A downstream industry adds a concentration of zinc that would be nontoxic in the absence of copper. The stream water is soft, naturally acid, and temporarily has its oxygen content reduced in passage through a lake supporting a heavy algal bloom. All factors contribute to a kill at a smaller concentration than would otherwise be possible. If a fish kill occurs below the second plant, what caused the kill and who is responsible? Must we indict Mother Nature as well as both industries, since all have changed the composition contributing to the kill? Or, if no kill would have occurred if the oxygen had not been reduced, does this confer immunity to the industries? How much time must be spent to evaluate the possible effect of other components in the wastes and water? This may sound ridiculous, but can a satisfactory conclusion be developed on the basis of a less thorough investigation?

There seems to be a need to consider a different approach in which absolute values shall be established for such factors as oxygen and pH. In the absence of toxic compounds in a quantity that will cause a kill without standards violation, a kill occurring during such a violation shall be ascribed to pH or oxygen.

The addition of any chemical or waste that can be shown to change a previously nontoxic complex to a toxic one shall be considered to cause the kill, even though substances in the water act to increase the toxicity greatly. This approach now forms the basis for the use of bioassays to establish the source of a kill when additional supplementary evidence is not obtained. The stream itself is a large-scale bioassay, but the location of a kill is only circumstantial evidence, since contributions from unknown sources are always possible.

Where this approach is justified legally, the use of bioassays to substantiate the findings would more nearly equalize the work load of the chemist and biologist.

Most fish kills are the result of a spill, or abnormal discharge, that exceeds the concentration tolerated by the species present for a short time. This is short-term or acute toxicity. Chronic toxicity may distribute the mortality over such a long period of time that no kill is noticeable. The 24-, 48-, and 96-hour TL_m's are unsatisfactory for use with most fish kills, and a time-concentration toxicity curve based on much shorter intervals should be used. In our laboratories we have used the logarithmic mean of time at each concentration for the construction of such curves. This reduces the effect of the inclusion of an abnormal fish by a factor equivalent to the number of fish tested at that concentration. It may be necessary to construct additional curves covering the effect of other variables, such as oxygen, pH, alkalinity, or hardness of the water, and the effect of other chemicals that may be present. Slope and curvature may vary in the same species during different periods of the year and with the previous history of the water.

The discharge causing a kill may be of short duration. Initially diluted in mixing with the stream, it will be further diluted from the front and back in passage downstream. Depending upon the duration of discharge, the central portion will retain the concentration of initial dilution for varying periods of time. The toxic agent and the source cannot be positively identified unless stream samples can be shown to be lethal. Early observation of the kill and speedy collection of samples is necessary for any evaluation. In New York we try to solve this problem by requesting the public to call the local game protector as soon as a fish kill is observed. He investigates, collects samples, notifies the pollution laboratory, and obtains any further instructions.

Fish kills may be grouped in two major categories, those caused by oxygen deficiency and those due to toxic compounds.

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† Because of the large number of references involved, no attempt has been made to list authors publishing on various phases of the problem of interaction among various chemicals. Those interested in additional references are referred to the reviews of the literature by the Water Pollution Control Federation Research Committee published in the Journal of that Federation and the former publications of this same organization.

KILLS CAUSED BY OXYGEN DEFICIENCY

Fish kills that are brought about by oxygen deficiency are usually the most plentiful and, in the absence of toxic substances, the most easily solved. The character of the district above the point of kill often excludes the chance of toxic substances, from other than the suspected source being present. The nature of the operation at the source may further support the absence of toxicants.

Graphs depicting the mean lethal oxygen concentration for one of the species involved in the kill are of great value in establishing the cause to be oxygen deficiency. A closed-jar procedure in which the respiration of the fish reduces the oxygen to lethal or near lethal concentration is adequate for the establishment of these curves. Observation has confirmed that there is close correlation between the laboratory data and field conditions producing the same effect. A series of concentration-temperature curves for some of the species frequently involved in kills in New York State (Burdick et al., 1954, 1957) is presented in Figure 1. For the sake of clarity, the range of variation shown in the original papers is not presented here, although it may be of significance in partial kills. Two curves are presented for rainbow trout that show small, but consistently increased resistance of wild fish over hatchery stock. This may be due to physiological differences. A similar comparison of lake- and stream-reared smallmouth bass indicates a higher resistance of the lake fish to reduced oxygen. If possible, use of spawning fish should be avoided in such experimentation since the oxygen requirements of some species are higher and the fish exhibit extreme variability.

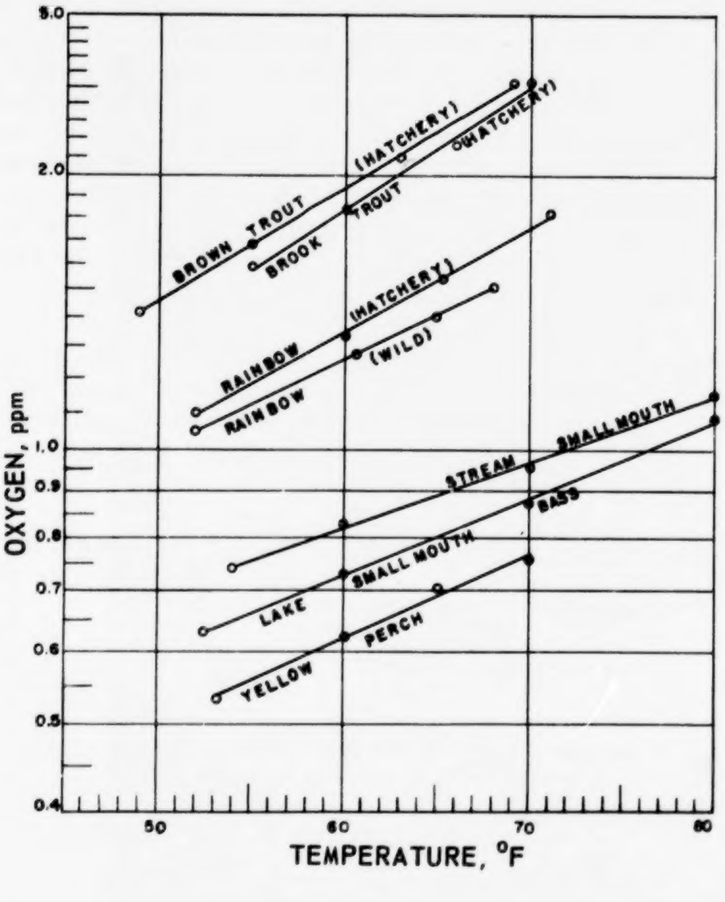

Figure 1. Mean lethal oxygen levels with closed-jar method.

The recommendations of Doudoroff et al. (1951) for acclimatization to temperature and water, feeding schedule, and other requirements for a valid bioassay should be followed unless it can be shown that failure to do so will introduce no error. Exception can be made in the volume of experimental solution. It has been found in our laboratories that no significant difference occurs in reaching the endpoint in 2 to 2-1/2 hours compared with more than 6 hours.

If no such data are available for any species found in the kill or if confirmation of these curves is desired, additional data should be obtained. While fish and water can be transported to the base laboratory, modification to a field method can save time and effort. Such a field method to provide the needed comparisons is tentatively suggested here. This is based on theory and has not been field tested. It should also evaluate the interaction of those chemicals showing increased toxicity with reduced oxygen.

PROCEDURE FOR FIELD ASSAY OF LETHAL OXYGEN CONCENTRATIONS

Water is brought from points where the stream is in natural condition to just above the entrance of the suspected waste. The containers should be set in the water and brought to the same temperature. If upstream discharges are present or suspected, water from this location should also be used. Jars similar to those described by Burdick et al. (1954) are convenient. The size should permit the endpoint to be reached in not less than 2 and preferably 4 to 6 hours. Experimental fish can be taken from the stream, with care to handle and excite them as little as possible. The stream can be used as a temperature bath, recording temperatures at 1/2-hour intervals. If the temperature fluctuation is extreme, all jars should be started within a short time period. From 5 to 10 jars from each water should be enough to determine the endpoint. A single fish should be used in each jar.

INTERPRETATION OF RESULTS

Any significant quantity of toxic material acting synergistically with low oxygen should produce a higher oxygen at endpoint because of the decreased respiration time. This can be tested statistically and, if significant, confirm the presence of a toxic compound. Toxicity from an upstream source can be ignored if the differences are slight.

If the oxygen was less at the point of kill than the requirement of the species at that temperature, the suspected source is the probable cause. If the oxygen was higher, the sample either does not correspond with the conditions at the time of the kill or the effluent also contains toxicants. A modification using dilution with upstream water could decide this point.

KILLS CAUSED BY TOXIC COMPOUNDS

Industrial wastes and agricultural and pesticide chemicals are the main causes of this type of kill. After collection of a sample assumed to contain the toxic substance or complexes causing the kill, the sample must be proven toxic. This can be done by routine bioassay in the majority of cases.

A chemical determination of the toxicant requires information on the process chemicals, products, and by-products involved in industrial manufacture, or the chemical in use, the amount, and area in which it is used with the agricultural and pesticide chemicals. A decision must then be made on the chemical or chemicals to be determined. For many chemicals satisfactorily sensitive methods may not be available at the toxic or sublethal level. Proper methods for concentration may also be lacking. One of the greatest needs in the pollution field is for more sensitive procedures for determining many chemicals.

It is an obvious impossibility to test any effluent for all possible chemicals that might enter into interaction in the toxicity. Such a procedure would have no place in field operations in pollution. It is believed that no mathematical models have yet been proposed to cover interaction of systems of even two to three chemicals, with variations of oxygen, pH, and other factors, that would enable it to be applied to field situations. Even if such a model were available, other chemicals would have to be excluded before it could be applied.

Where a method is available to test for a toxic chemical, and the concentration is found to exceed the lethal level for the pure compound, interaction with other chemicals cannot yet be excluded. Responsibility can be fixed by bioassay if an equivalent concentration of the pure compound fails to show an increased or decreased death time in comparison with the stream samples.

If the death times are different, the source of the interaction might be determined by the closed-jar assay as suggested under kills from oxygen deficiency. Further work is unnecessary when the source can be confined to a single effluent.

The wording of the law would decide any further action to be taken if the complex is found to be derived from multiple sources.

THE USE OF POST MORTEM ANALYSES IN THE DETERMINATION OF THE CAUSE OF FISH KILLS

Post mortem analysis to determine the cause of death in man has been so widely publicized that probably every biologist has dreamed of using a similar procedure to determine the cause of death in fish. Possibilities are indicated in the reported work of Carpenter (1927) in revovery of heavy metals by acid stripping of fish. DDT can be recovered from the stomach contents of fish allegedly killed from eating floating insects carrying DDT. Cyanide is so frequently determined in man that it is natural to seek a similar procedure for fish.

One of our laboratories spent over 6 months in preliminary work on such a procedure several years ago. Much of the work was chemical in nature. Both fish tissue and the gall bladder contents gave indications of heavy cyanide content by the Aldridge method (Aldridge, 1945). Baffles were indicated to avoid possible entrainment in the distillate when an acid distillation procedure was involved.

At relatively large concentration the cyanide content varied directly with the exposure concentration. The recoverable cyanide fell over a 24-hour period if the fish were allowed to stand in fresh water. This was particularly true with the use of the gills. The data are presented in Figure 2. The decrease was somewhat less noticeable with the combined tissues of heart, liver, and spleen presented in Figure 3.

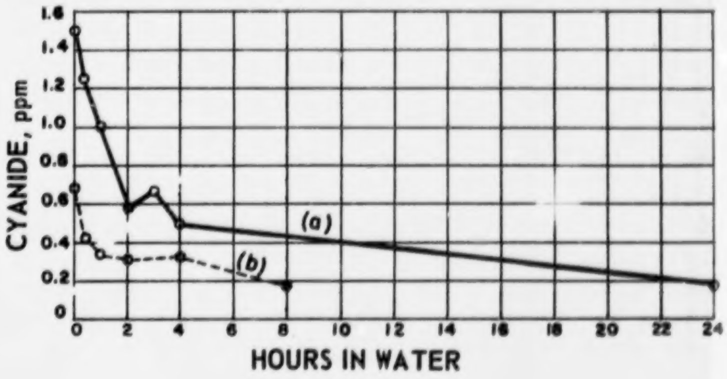

Figure 2. Loss of CN⁻ in water as shown by recovery from gills of fish killed in: solutions containing (a) 2.8 to 3.0 and (b) 0.60 to 0.62 ppm as CN⁻.

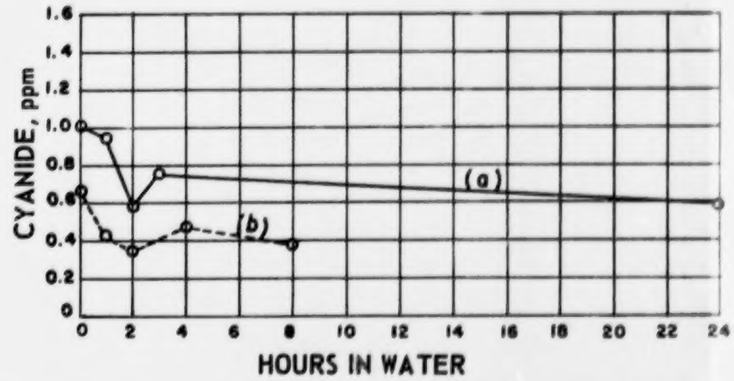

Figure 3. Loss of CN⁻ in water as shown by recovery from heart, liver, and spleen of fish killed in: solutions containing (a) 2.8 to 3.0 and (b) 0.60 to 0.62 ppm as CN⁻.

The use of liver and spleen (Figure 4) appeared to restrict the variation to the range of individual variation of the material.

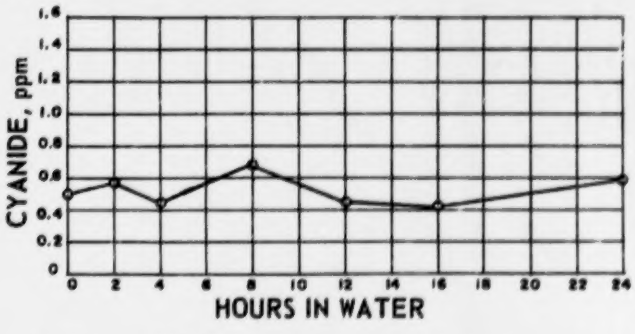

Figure 4. Loss of CN⁻ in water as shown by recovery from liver and spleen of fish killed in solution containing 0.67 ppm as CN⁻.

The cyanide found in unexposed fish was highly variable. At the higher levels it occasionally approached that found as minimal levels in fish exposed to cyanide. Losses on standing in water raised problems in evaluation not easily solved. The studies of Doudoroff (1956) and Lipschuetz and Cooper (1955) on metal cyanide complexes raise further questions on the validity of such a determination. These papers indicate increased toxicity over that of KCN for some metal complexes, reduced toxicity in others. These relationships are not completely explainable on the basis of total cyanide, removal of cyanide by the metal complex, or the availability of HCN, but in some cases must involve synergism of the metal.

The work of Burdick et al. (1958) on the toxicity of cyanide to brown trout and smallmouth bass at two oxygen levels indicates that reduction of oxygen will greatly increase the toxicity of cyanide. The curves are presented in Figure 5 for purposes of comparison. The curves are not parallel and indicate a greater effect in the intermediate concentrations. This raises an additional problem in the evaluation of the quantity of cyanide producing death based on a post mortem analysis. It was concluded that there would be such a limited usefulness for such a method that the work has been abandoned.

With the great number of possible contaminants to be found in water that can enter into interaction to produce death, the use of any post mortem procedure for the accurate determination of the cause of death of fishes seems theoretically impossible.

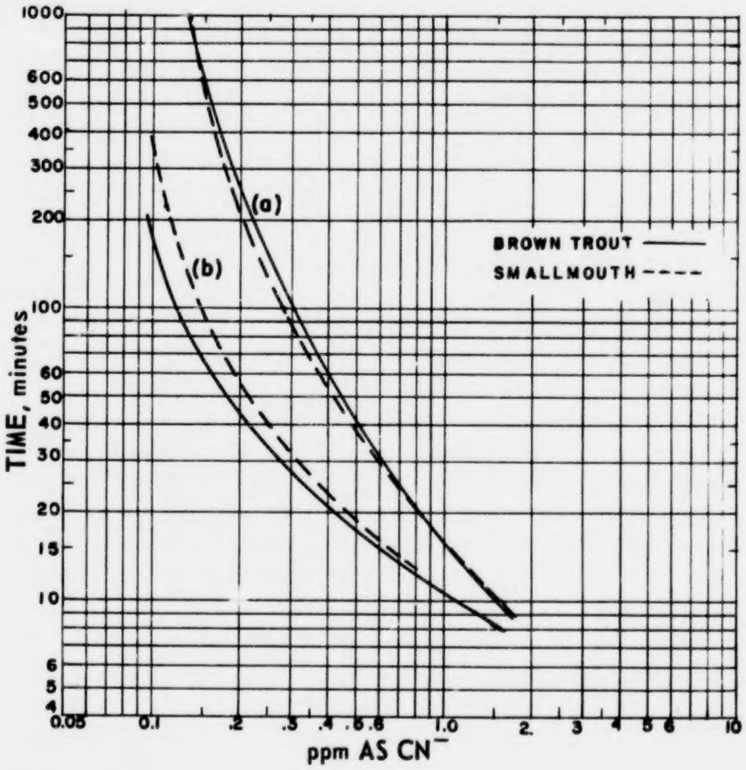

Figure 5. Effect of oxygen on the toxicity of cyanide.
Brown Trout: Oxygen ppm - (a) 9.7 (b) 5.1 to 5.3
Smallmouth: Oxygen ppm - (a) 9.0 (b) 4.1 to 4.2

Man's environment is much more stabilized and loss or the further intake of toxic substances by ingestion or respiration is less likely than in the lower vertebrates where circulation often continues for some time after death. In fish, the intake of most toxic materials is through the gills, a semipermeable membrane through which exchange may occur from one liquid medium to another quite readily. The problem in fish is thus much more complex and less readily solved than in the case of man.

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AN EXPERIMENTAL ANALYSIS OF THE FACTORS RESPONSIBLE FOR PERIODIC FISH MORTALITIES DURING WINTER IN BUSHVELD DAMS IN THE TRANSVAAL, SOUTH AFRICA

*B. R. Allanson, M. H. Ernst, and R. G. Noble**

INTRODUCTION

The highveld-bushveld transition region in the Transvaal, South Africa, lies at an altitude of between 3,800 and 4,500 feet above sea level, and the dry winter season is accompanied by frosts and extended cold periods. Allanson and Gieskes (1961) have reported some of the limnological changes brought about by these cold winters in Hartbeespoort Dam, 18 miles west of Pretoria. They have reported water temperatures of 13°C during July and August, months when water temperatures are always lowest. It is usually during these months that extensive fish mortalities, particularly of *Tilapia mossambica* Peters, an introduced cichlid, are reported. In recent years considerable speculation about the cause has been made; the discharge of toxic effluents from factories and local authorities in the catchment of these dams has often been blamed. Sport fishermen have put forward another viewpoint and suggested that temperature might be an important factor that should be studied in some detail. To this end the National Institute for Water Research cooperated with the biologists of the Department of Nature Conservation, and an attempt was made at an experimental level to understand the effect of low temperature upon *T. mossambica* in an endeavor to decide whether or not to support this view.

The following experiments were carried out after sighting studies:

1. The effect of low temperature on the behavior of fish on direct transfer into waters of low and high total dissolved solid (TDS) content during a set exposure time.
2. The estimation of an "effective concentration 50" (EC50) using percentage collapse as the dependent variable. This study was done only in water of high TDS.
3. The estimation of a "lethal concentration 50" (LC50) using percentage mortality as the dependent variable in water of low and high TDS.

This study has formed a second part of a larger study on the effect of temperature upon *T. mossambica.* The first part dealing with the tolerance and resistance of this species of fish to high temperature is awaiting publication.

METHODS

The Lowveld Fisheries Research Laboratories supplied 1,200 young *T. mossambica,* measuring beteen 10 and 12 cm in length. The fish in the sample supplied were between 6 and 8 months old and were collected from open dams in which the temperature varied about 25°C. This population was randomized on arrival in the laboratory among six holding tanks. Water was continuously supplied to the tanks from a borehole and, after ion exchange to remove an excess of iron in solution, had the following composition:

Table 1. THE COMPOSITION OF BOREHOLE WATER SUPPLY TO THE HOLDING TANKS AFTER ION EXCHANGE

Conductivity, μmhos	22.5
pH	6.5
Total dissolved solids as ppm	19.0
Nitrate as ppm NO_3	0.20
Phosphate as ppm PO_4	0.01
Alkalinity bicarbonate as ppm $CaCO_3$	8.2
Sulphate as ppm SO_4	0.9
Chloride as ppm Cl	0.8
Total hardness as ppm $CaCO_3$	7.1
Ca hardness as ppm $CaCO_3$	4.9
Mg hardness as ppm $CaCO_3$	2.4
Sodium as ppm Na	0.6
Potassium as ppm K	0.3
Iron as ppm R_2O_3	0.2

The pH was kept roughly constant between 6.9 and 7.0 by strong aeration of the holding tank water, which was kept at 20°C ± 0.1°C.

The fish were maintained under these conditions for 14 days before the investigation started. The investigation lasted for 3 months; thus, the last group of fish to be used had lived under these conditions for 14 weeks.

For each phase of the investigation the total number of fish in any one holding tank was randomized between 10 buckets from which 6 buckets were randomly taken. As far as possible 20 fish were used in each of six experimental or chill tanks, each of which had a capacity of 2 cubic feet. The temperatures in the experimental aquaria were kept constant (± 0.1°C) by modified "Techne Tempunits," and the water was fully saturated with oxygen (7 ppm) by aeration at approximately 2.5 liters/minute.

Previous sighting experiments had shown clearly that it was not possible to treat the investigation on a time-mortality basis as Brett (1952) had done using salmon fry, because no clear-cut indication of death such as gaping or permanent erection of the pectoral fins was available in *T. mossambica*. The whole investigation, therefore, rested on response to "doses" of temperature for given exposure times.

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In each of the three phases of the study, fish held at the temperature conditions described above were acclimated to 25°C for 2 days at an adjusted pH of 7.0. Earlier unpublished studies on the tolerance and resistance of *T. mossambica* to high temperatures have shown that this cichlid species is capable of very rapid acclimation to high temperatures. From these data it was known that 2 days' exposure to 25°C from a holding temperature of 20°C was quite sufficient to effect complete thermal acclimation to the higher temperature. The technique of direct transfer to the experimental temperatures was used.

THE PATTERN OF RESPONSE ON DIRECT TRANSFER TO LOW TEMPERATURE

A sighting experiment at the start of this study indicated that the temperature at which responses to chilling occurred might vary with the total dissolved solid (TDS) concentration of the water used. Thus, in the further design of the investigation an attempt was made to repeat experiments in both borehole water and tapwater supply, the latter having a TDS concentration of 130 ppm, which is at least six times that of the former.

WATER LOW IN DISSOLVED SOLIDS — Borehole supply, TDS 19 ppm.

In Figure 1 the variation in pattern of collapse is clearly shown in relation to temperature and time. The behavioral categories of normal individuals were: (a) Active, (b) quiescent and sporadically active, (c) quiescent, and (d) moribund but upright. The categories of collapsed individuals were: (a) Lying on right or left side but with occasional sporadic activity and (b) no recovery at all from lying on right or left side.

Between 10.5° and 11.5°C there was clear evidence of an initial high incidence of collapse, followed by a gradual recovery at 10.5° and 11.0°C. Recovery at 11.5°C was more rapid and led to a higher incidence of recovery by the individuals in the sample. At all three temperatures there was a period of maintained recovery lasting between 120 and 150 minutes. This was followed by a variable rate of collapse that depended on temperature and was most rapid at the lowest temperature, 10.5°C.

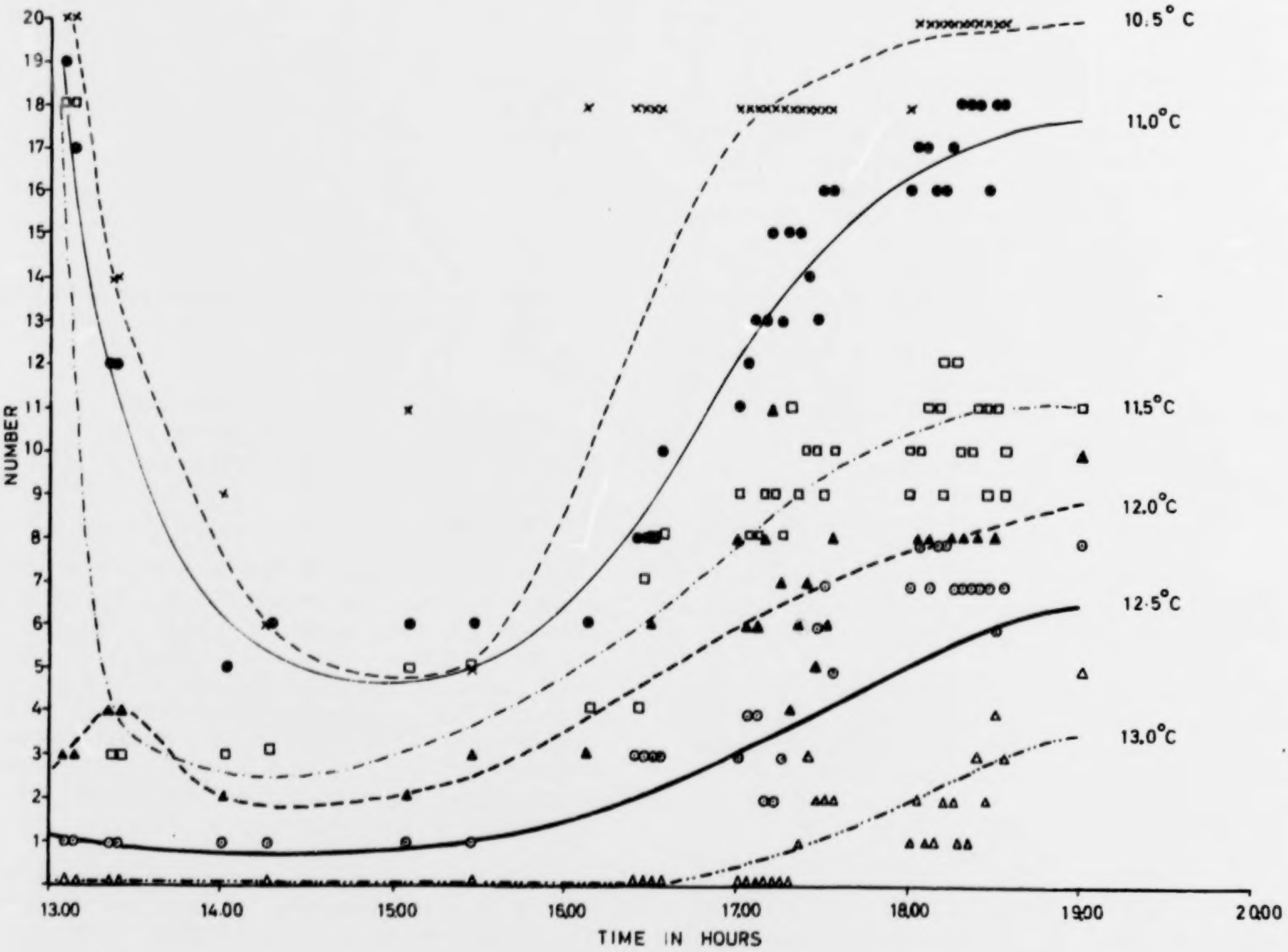

Figure 1. The Pattern of collapse for *T. Mossambica* in water of low dissolved solids.

At temperatures above 11.5°C the initial high incidence of collapse was not found, although two or three individuals did collapse immediately upon transfer. As Figure 1 shows, this number decreased until at 13.0°C no initial collapse of any of the fish used was observed. Only after 180 minutes at these temperatures did the incidence of collapse increase with time.

Because there was in all cases either a recovery from initial collapse on transfer for the majority of individuals used or a slow increase in incidence of collapse only after 180 minutes' exposure to the temperature of the experimental aquaria, two components of the changes brought about by cold as described by Pitkow (1960) were considered evident. There was a recovery from the shock that occurred between 10.5° and 11.5°C. This was followed eventually by collapse into secondary chill coma from which there was no recovery on transfer into warmer water. There was no evidence of primary chill coma since recovery occurred, and as Pitkow has pointed out, a primary requisite of primary chill coma is that the organism does not recover unless transferred to warmer water.

A further experiment designed primarily to obtain an estimate of temperature EC50 where six random samples of 20 fish were exposed for 1,000 minutes to temperatures from 13.0° to 15.5°C provided further evidence that supported the existence of a secondary chill coma in *T. mossambica*.

WATER HIGH IN DISSOLVED SOLIDS — Tap water supply, TDS 130 ppm.

The data available are given in Figure 2. Unfortunately these data are not as detailed as obtained under (1) above. A similar trend is found in the response of fish to immediate transfer into water of high dissolved solid content as is found in the immediate transfer into water of low TDS.

Lower temperatures and longer exposure time, however, were necessary to obtain similar responses in water high in dissolved solids.

In addition, the data given in Figure 2 indicate the temperature at which primary chill coma was found and a possible gradation into a shock-recovery-secondary chill coma sequence. At a temperature of 11.5°C this sequence appeared to be replaced by

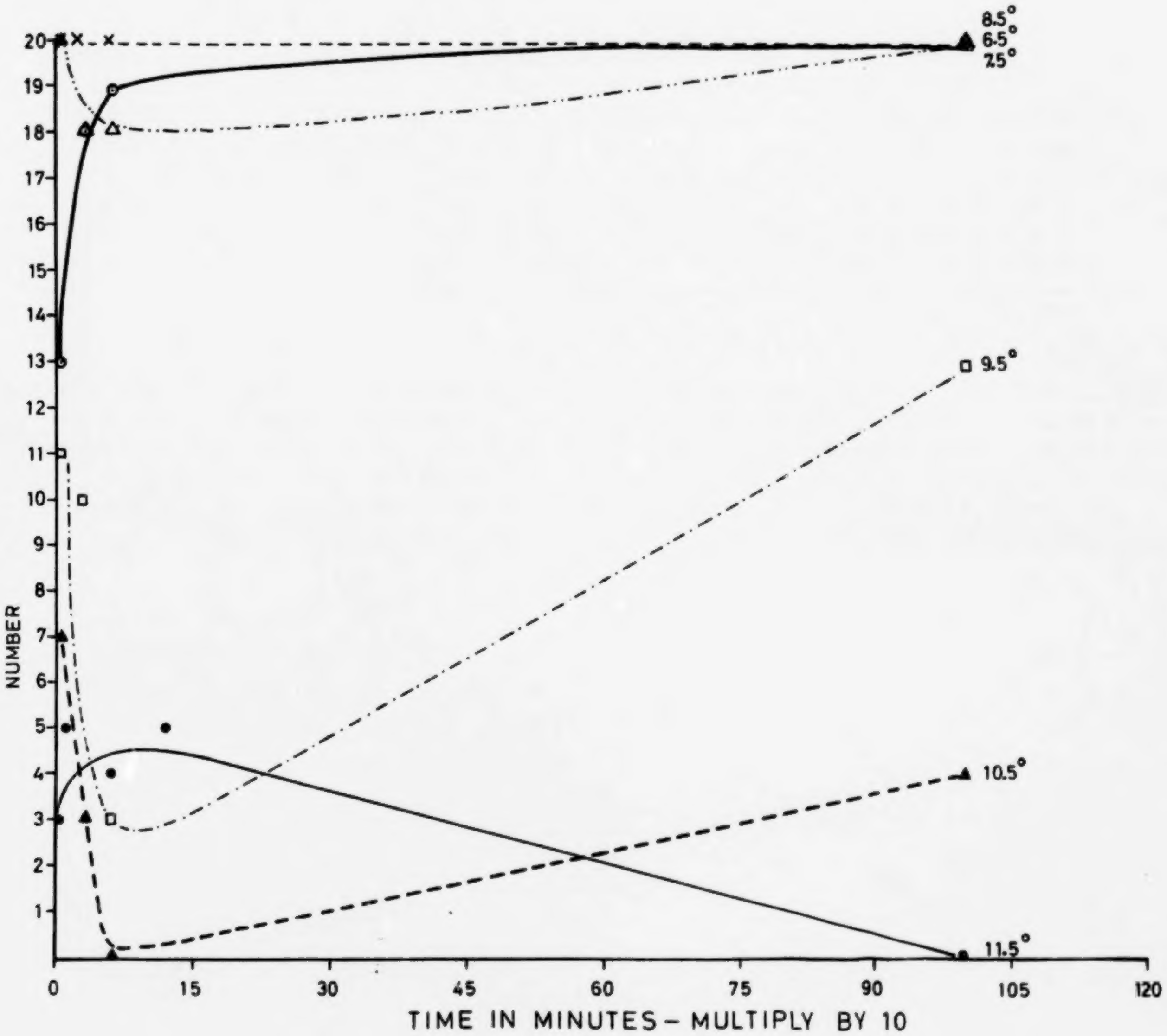

Figure 2. The pattern of collapse for *T. Mossambica* in water of high dissolved solids.

low incidence of collapse due to shock and eventually lead to a complete recovery of all the fish in the sample used.

EFFECTIVE CONCENTRATION 50 USING PERCENTAGE COLLAPSE AS THE DEPENDENT VARIABLE

The preceding analysis of the pattern of response on direct transfer to water of low temperature leads logically into an investigation of the usefulness of collapse as a metameter from which an EC50 could be estimated. The estimation of an EC50 was also supported by field observations made in winter when numerous juveniles of *T. mossambica* were found lying on the bottom of the shallows in the reservoirs of this region.

Because the waters of these irrigation reservoirs had concentrations of TDS nearer to the tapwater supply in the laboratory than to the water of the borehole, the estimation of an EC50 for this species was made by using tapwater. The experimental data are given in Table 2, which combines the data from three random groups of fish.

LETHAL CONCENTRATION 50

The pattern of mortality after 1,000 minutes and the pattern as measured by the number of individuals that did not recover after transfer to water at 18°C for 6 hours were not found to be homogeneous either in tapwater or in borehole water. This was due mainly to the small numbers (20 individuals) used at each experimental temperature. In Figure 4, however, data from two random blocks of fish are combined and suggest that a split probit of mortality may be expected. Thus, mortality from shock may be separated from that due to primary chill coma after 1,000 minutes' exposure.

The shock effect following immediate transfer to cold temperatures is obviously of great importance in the design and interpretation of experiments and results and is one that Brett (1952) clearly indicated in his time mortality studies on salmon fry.

From these data, however, it was not possible to obtain an estimate of the LC50 in tapwater supply and it is doubtful at this stage whether the LC50 has any tangible ecological significance.

Table 2. THE INCIDENCE OF COLLAPSE IN *T. MOSSAMBICA* IS EXPRESSED AS A PERCENTAGE OF THE NUMBERS EXPOSED AT EACH OF THE (FOLLOWING) TEMPERATURES INDICATED AND FOR THE TIME GIVEN. THE FISH WERE ACCLIMATED FOR 2 DAYS IN TAPWATER WITH TDS 130 ppm AT 25°C and pH of 7.0

Temp, °C	8.0	8.5	9.0	9.5	10.0	10.5	11.0	11.5	12.0	12.5	13.0	13.5
6 min.	100	65	55	55	55	35	30	15.8	5	25	0	0
30 min.	100	90	85	50	55	15	16.2	26.3	5	0	0	0
1,000 min.	100	100	95	65	45	20	5	0	0	0	0	0

An empirical probit plot of the collapse data after 1,000 minutes is given in Figure 3. The fitted probit line was computed according to the method of Finney (1952) and the experimental data are regarded as homogeneous; the graphical estimate of the EC50 is 9.9°C, and that by calculation, 9.90°C.

It should be noted that, so far, it has been possible to induce true secondary chill coma and, therefore, mortality in tap water only between 10°C and 11°C. Above this temperature and up to 15.5°C an exposure of even 4,000 minutes did not cause any change in the survival of the fish used.

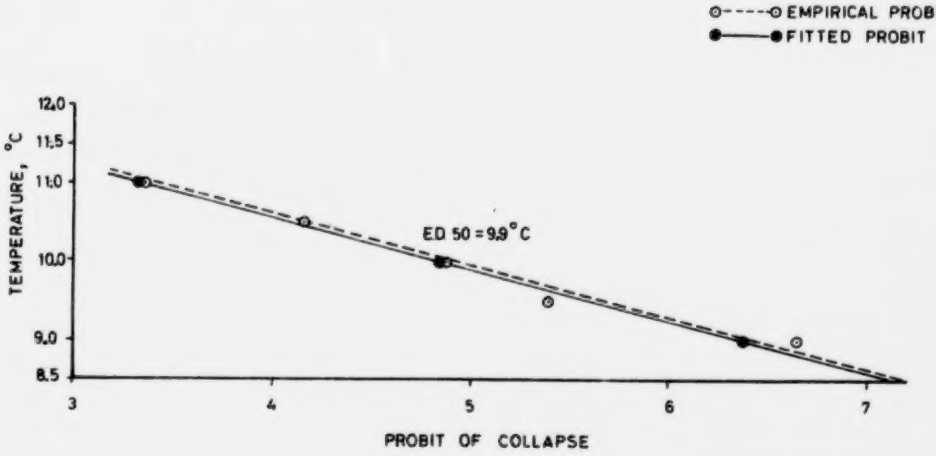

Figure 3. The empirical probit and fitted probit of collapse for *T. Mossambica* after 1,000 minutes' exposure.

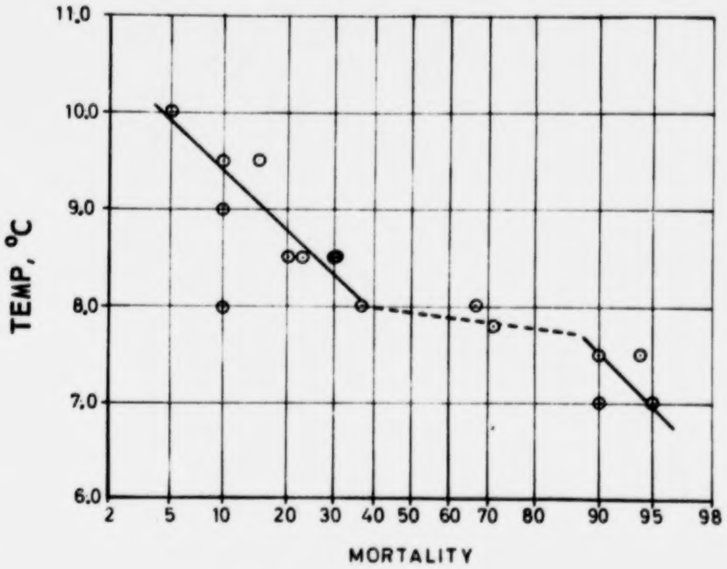

Figure 4. The mortality of *T. Mossambica* at low temperatures in water high in dissolved solids at pH 7 and after 2 days' acclimation at 25.0°C.

A POSSIBLE EFFECT OF THE CONCENTRATION OF TOTAL DISSOLVED SOLIDS UPON THE LC50

Pitkow (1960), working on the characteristics of cold tolerance in the guppy, *Lebistes reticulatus,* reported that increasing the osmotic pressure of the water used had no significant effect upon the guppy's cold tolerance. Throughout this investigation a borehole water supply with TDS of 19 ppm was used as far as possible in parallel with tapwater that had a TDS of 130 ppm. As a result there appeared clear indications that change in the tonicity of the water supply certainly had an effect both upon the LC50 and EC50, although it was not always easy to repeat the investigation on remaining random groups after the initial striking difference between mortality was observed. These data are given in Table 3.

This lack of reproducibility might be due to variations in conditions during the holding of fish under laboratory conditions for a long time. This was necessitated because this investigation was carried out during our winter period when no additional stocks of fish could be obtained from the Lowveld Fisheries Research Station. It is considered, however, that sufficient data are available for an effective experimental design.

CONCLUSION

Finally, the results obtained from this investigation indicate quite clearly that low temperature is certainly an important factor for fish mortality in the reservoirs in the highveld-Bushveld transition. How the effectiveness of this factor changes with changes in other factors such as dissolved oxygen, pH, and low concentration of toxic substances obviously requires further study.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation of the kind cooperation of Dr. G. L. Lombard, Officer in Charge, Lowveld Fisheries Research Station. This paper is published by permission of the South African Council for Scientific and Industrial Research and the Directorate of the Transvaal Department of Nature Conservation.

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Table 3. THE POSSIBLE EFFECT OF CHANGE IN DISSOLVED SOLIDS CONCENTRATION ON THE MORTALITY OF *T. MOSSAMBICA* EXPRESSED AS A PERCENTAGE AFTER 1,000 MINUTES' EXPOSURE AND AFTER 6 HOURS IN WARM-WATER RECOVERY TANKS AT 18°C.

Temp, °C	13.0	13.5	14.0	14.5	15.0	15.5
H_2O with low dissolved substances	90	100	90	65	55	40
H_2O with high dissolved substances	0	0	0	0	0	0

DISCUSSION

Most of the investigators in pollution biology are very interested in good post mortem methods that will give reliable answers as to the cause of death. Such methods would lessen the burden of proof on the prosecution in cases following fish kills. Frequently bioassay techniques are not practical or cannot be used to secure the necessary data. There has been only limited work up to the present on the development of such autopsy techniques. Many difficulties have been encountered. These include difficulty in analysis of many of the offending compounds, little or no knowledge of the mode of action of these materials on aquatic life, and pitfalls inherent in the organisms themselves such as autolysis and back leaching.

Methods that have been developed include a method for cyanide, by the laboratory at Pitlochery, Scotland; histological determinations on gill damage for several of the heavy metals, and methods for the determination of DDT in tissues. All of these methods have certain drawbacks and have been useful only in selected cases.

Two remedies were proposed for this problem. They were better liaison with industry and all potential polluters to give previous information on potential pollutants, i.e., bioassay before the fact, and a determined effort to develop autopsy techniques along the lines of those used in human medicine today.

The session concluded with a spirited discussion of the problems encountered in the rearing and harvesting of *Tilapia sp.* in the United States and in temperate and equatorial Africa.

THE ARTIFICIAL EUTROPHICATION OF OUR WATERS

Eugene A. Thomas, Chairman *

THE EUTROPHICATION OF LAKES AND RIVERS, CAUSE AND PREVENTION †

Eugene A. Thomas

INTRODUCTION

As we all know, preserving the purity of rivers and lakes in all industrial countries and wherever many people live close together has become one of the important tasks of men in recent decades, especially since the end of the Second World War. In many countries, it was recognized a century ago that the pollution of lakes and rivers should be prevented and, even at that time, laws and regulations were made to preserve the purity of the water. In Switzerland, for example, on 10 December 1876, the excellent law concerning public hygiene and food inspectors came into force and on 1 June 1881 the explicit Zürich cantonal regulation regarding the preservation of purity of water was issued. When this regulation was passed, the director of the medical department laid down conditions that had to be met for running water at 50 meters from a sewage inlet and for standing water at 100 meters from such an inlet. These conditions referred to potassium permanganate consumption (less than 60 mg/liter), nitrogen in a soluble organic compound (less than 1 mg/liter), soluble metal compounds of lead, copper, etc., harmful to health (less than 2 mg/liter), arsenic in any form (less than 0.05 mg/liter), active chlorine becoming free on acidulating with sulphuric acid (less than 1 mg/liter), hydrogen sulphide or sulphide decomposable with carbonic acid (less than 1 mg/liter), free acid (in 1 liter less than is neutralized by 10 ml normal alkali), free alkali (in 1 liter less than is neutralized by 10 ml normal acid), and so little coloring substance that a 10-centimeter layer of water in a white container did not show any definite color in daylight. All these regulations, however, were far too seldom enforced.

Today there is greater understanding among responsible authorities and by many people of the need for purifying sewage. In many cases, however, it is not easy even for an expert to determine how extensively sewage must be purified before it may be discharged into rivers or lakes. Biological problems are of especial importance here. After purification, sewage that is ready to be discharged may be absolutely nonpoisonous and largely freed of organic substances and yet it can produce phenomena that are undesirable in many respects. The following exposition deals with these phenomena and the possibilities of preventing them.

I should like to thank the American Department of Health, Education, and Welfare, especially Dr. Clarence M. Tarzwell, Chief of Aquatic Biology, Research Branch, Division of Water Supply and Pollution Control, for the invitation to attend this Third Seminar on Biological Problems in Water Pollution. I am also grateful to the American Society of Limnology and Oceanography, the Chancellor of Zürich University, and the Board of Health of the Canton of Zürich for supporting the journey to America.

I. The original state of lakes and rivers

A. The original state of lakes

Since the beginnings of limnological research, considerable interest has been shown in the morphology of lakes. In the "Handbuch der Seekunde" (Handbook of Information on Lakes) by F.A. Forel (1901), the section on morphology comprises one-seventh of the book. Since then it has very often become necessary to deal especially with lake volume when interpreting results of research on lakes. In northern Switzerland, for example, all lakes of 20 meters' depth or more with at least 500,000 square meters of surface area belonged to oliogotrophic types until a few decades ago, as research on the mud sections has shown (Thomas, 1949). Today most of these lakes are in a eutrophic state as a result of artificial fertilization. For the following comments, lakes are divided into two morphologically defined categories: the larger lakes (at least 20 meters deep and with a minimum surface area of 500,000 square meters) and small lakes (less than 20 meters deep and with a surface area of less than 500,000 square meters).

1. The original state of larger lakes

Today there is fortunately still a number of large lakes that have completely preserved their original state — or practically so. Thanks to their depth, they have a large quantity of hypolimnic water at their disposal. The blue or greenish-blue water is clear and transparent and its content of phosphorus compounds is so low that plants cannot make use of the available nitrates. There is only a quantitatively moderate development of phytoplankton organisms even in the surface water, and littoral plant production is also slight. The high permeability of light prevents the formation of a distinct thermocline and provides the plants with good conditions for development even at great depths.

The lime content and the oxygen content of these lakes are approximately the same from the surface down to the greatest depth, and there is no disappearance of oxygen in deep water. Ammonia, iron,

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manganese, and hydrogen sulphide are also absent there. Littoral plant growth and phytoplankton production are very slight during the whole year, in contrast to the deep fauna, which is comparatively rich. Fish present are mainly Coregonen.

2. The original state of small lakes

In a small lake without human influence the tributaries and wind bring far more dead leaves, pollen grains, insects, and other organisms per square meter of lake surface and per cubic meter of volume than in a large lake. These dead organisms and parts of organisms decompose in the water and release fertilizers, resulting in the increased development of plankton and shore algae. Because of this, oxygen disappears and ammonia, iron, manganese, and hydrogen sulphide appear in the deeper water during summer stagnation, without human influence.

B. The original state of rivers

In rivers that are not contaminated by sewage, we generally expect clear, bluish water; only in boggy regions is it brownish. The stones on the river bed are only very slightly overgrown with algae, if at all. The concentration of oxygen is neither very high nor very low. Such rivers are suitable for development of fishing and also for drinking water supplies or for swimming.

II. The consequences of pollution by sewage

A. The consequences of pollution in lakes

Unpurified sewage is noted by the turbidity and discoloration of the lake water and, in serious cases, by the presence of paper and other solid substances and also of oil. Bacteria as well as plankton and shore algae develop rapidly. Small lakes react quickly to the introduction of sewage. Oxygen rapidly disappears in the hypolimnic water. Complete disappearance of oxygen can also occur in large lakes in varied places: (a) In the water just above the mud on the bed, (b) in the lower part of the metalimnion, and (c) in places on the shore where masses of algae have been driven and have then rotted (Thomas 1955a, 1960a). The unfavorable proportion of oxygen in such lakes endangers or destroys the stocks of valuable Coregonen so that it is impossible to develop the lake for fishing. The preparation of drinking water from polluted lakes is rendered difficult, and swimming cannot be allowed in the proximity of sewage outlet pipes.

B. The consequences of pollution in rivers

The banks of rivers, in contrast to lakes, are very extensive in comparison with the surface. Aesthetically unpleasant conditions therefore annoy residents on the banks, fishermen, swimmers, and walkers on both sides of the river. In this respect I am thinking not only of papers, rags, etc., but also of turbidity and discoloration.

Changes in the biocoenoses of the river bed caused by sewage and the appearance of polysaprobic organisms such as *Sphaerotilis* are also strikingly visible; rapidly growing tufts of *Sphaerotilis* are continually broken off by the current and transported long distances. According to Kolkwitz and Marsson (1908, 1909) and Kolkwitz (1950), water must flow for a considerable distance before self-purification brings a more favorable state of biocoenoses; bacteria and other heterotrophic organisms recede in favor of algae, the oxygen content of the water increases and the condition of the water improves again.

River pollution makes obtaining drinking water and using river water for industrial purposes more difficult. Where ground water is fed through rivers, it can be rendered useless by pollution. Only unpolluted rivers are suitable for swimming. The pollution of a river means partial or total depreciation of the stream for fishing purposes.

III. The first results of purifying sewage

A. Results of purifying sewage in lakes

The opinion is very widely held today that sewage to be discharged into lakes and rivers should be purified not only mechanically but also biologically. Special purification to remove fertilizers is seldom used. The following ideas start, therefore, with the assumption that sewage discharged into lakes and rivers should be purified mechanically and also biologically. The method of sewage purification put forward prevents any direct visible pollution of the lake shores, with the exception of scum originating from detergents, about which I shall not speak here. From an aesthetic point of view it is especially important that the natural water of lakes from which supplies of drinking water are taken is not polluted by sewage.

The content of bacteria, particularly the *Bacterium coli* group, in biologically purified sewage is much less than in unpurified sewage. For this reason, water near the shores of a lake that receives no unpurified sewage is much more suitable hygienically for swimming.

B. Results of purifying sewage in rivers

The aesthetic and hygienic results of sewage purification for lakes are also applicable to rivers. In rivers, moreover, it is relatively easy to get rid of the heterotrophic living communities caused by unpurified sewage by simply purifying the latter. At the same time it is possible to improve the oxygen content of river water effectively and permanently, providing thereby more favorable living conditions for fish. The death of fish due to purification of sewage, can, however, occur.

IV. Conditions for permanent improvement of the state of rivers and lakes

Unfortunately, mechanical and biological purification of sewage cannot alone prevent the enormous development of algae and higher aquatic plants. Biologically purified sewage still contains some substances that actively further the growth of certain green plants. What kind of substances are these?

In 1945 and later, I grew phytoplankton cultures both in lake water and in waters to which nutritive substances were added. These tests showed that the content of nitrogen and phosphorus compounds in surface water of eutrophic lakes varies considerably in the course of the year whereas the potassium content is more constant. Because of the dominating position of nitrogen and phosphorus compounds, the importance of trace elements or of growth substances and other organic substances was pushed into the foreground. For these reasons, I asked myself the following questions: Is the concentration of all other vital substances sufficient to allow correspondingly increased production of algae where the supply of nitrogen and phosphorus is artificially increased? Is a considerable part of the added nutritive substances that are used incorporated in organic form?

These questions were experimentally tested by adding surplus nitrates and phosphates to samples of water from 46 different lakes. We filled two to six Erlenmeyer flasks each with 300 milliliters of different lake water and sterilized them after stopping the flasks with cotton. We then added sufficient sterile nitrate and phosphate solution to each flask to bring the nitrate content to 20 milligrams per liter and the phosphate content to 2 milligrams per liter. Surface plankton from the mesotrophic Upper Lake Zürich served as the inoculum for these sterile nutritive solutions. Since the surface water composition in this lake is considerably different in summer than in winter, the water was tested once in summer and once in winter (Thomas, 1953).

The injected flasks remained for 2 months in diffused light and at room temperature. At the end of the test period, the plankton algae and tychoplankton had greatly increased in all the flasks. The nitrates had been completely used up in almost all the tests and the phosphates in some of them. The addition of nitrate and phosphate to water from lakes tested is, therefore, sufficient to further the growth of algae considerably. In other words, only nitrates and phosphates come into question as minimum substances in such lakes. Here minimum substance means the vital nutritive substance that checks the further development of algae by its deficiency in the water.

The mechanical/biological sewage purification used today, however, removes from sewage only a small part of the effective nutritive material for algae. Algae, being autotrophic organisms, can - as is well known - use such mineral substances to increase their own body substance and thereby increase the number of individuals.

The tests described prove only that the addition of nitrogen and phosphorus compounds to lakes and rivers is sufficient to stimulate some types of algae to greatly increased growth, which can, however, have very detrimental results.

A. Conditions for the permanent improvement of the state of lakes.

As has been mentioned, purification of the sewage will improve the waters on lake shores where sewage has a direct effect. Without doubt, however, the biggest problems arise from the proliferation of plankton algae and of shore algae (Thomas, 1960a, 1960b, 1961).

Plankton algae make the water turbid and discolored with unpleasant shades of green, yellow, brown, and violet. The floating layers of plankton algae are particularly disagreeable for swimmers, boaters, fishermen, inhabitants on the banks, and people walking nearby. Shore algae also are often unpleasant. On Lake Zürich in Switzerland, and on other lakes with a similar supply of fertilizers, the stones on the shore are covered with brown clumps of Diatoms several centimeters in extent even in early spring. At about the same time, Cyanophyceae (mainly *Oscillatoria limosa* Ag) overgrow all places where the surface consists of mud or lake chalk. On these blackish areas there is also a more or less plentiful growth of siliceous algae. Abundant CO_2 assimilation occurs when the sun is shining. Part of the released oxygen is held back in the entanglement of cyanophycian threads in the form of bubbles. These gas bubbles finally result in patches' larger than hand size being detached from the bottom so that they float on the surface and form repulsive flat cakes there that look like the skin of a toad. This unpleasant phenomenon is overcome only by rain and wind in April or May. The original biotopes of the "toad's skin" are then overgrown by other algae, particularly by extensive *Spinogyra* cushions, which then rise to the surface of the lake in summer and disfigure the shores.

In April or earlier, according to the weather, the stones near the shore become covered with a fine growth of *Ulothrix* threads. From about May until July all places on the shore are dominated by the green thread algae. At the same time *Cladophora* threads growing on stones, wood, reeds, etc., at depths of up to 3 meters, press up towards the surface of the lake accompanied by, among others, *Rhizoclonium*, *Microspora*, *Oedogonium*, *Mougeotia*, and *Zygnema*. The latter three may reach their maximum growth somewhat earlier. If the weather is favorable for the growth of *Cladophora*, the stony shore zones from 0 to about 3 meters deep are packed with the cotton masses of its threads. Oxygen bubbles released by assimilation raise some of the *Cladophora* pads to the surface of the water in sunny, calm weather, and yellowish-green layer forms, often many centimeters thick.

These tenacious, extensive masses of *Cladophora* that cover large areas of a lake are definitely annoying to everybody who comes in contact with them. But even worse is the annual damage to reeds. Formerly reeds swayed backwards and forwards in storms with the rise and fall of the waves, but today they are snapped off by the *Cladophora* masses in the border zone of the open water and are pressed below the surface. Later, the stalks that have been pressed down often produce adventitious shoots, which, however, die in the winter. The reed rhyzomes in the ground are so weakened by this process that they produce no stems the following year, or so few that they can offer still less resistance to the new masses of algae. This has resulted in a great decline in the growth of reeds on the shores of Lake Zürich, which is disadvantageous for fishing and regrettable from an aesthetic point of view. In addition to damaging the reeds, the *Cladophora* masses can cause an extreme lack of oxygen on the shores that is recognizable by the evaporation of hydrogen sulphide and other products of decomposition, and leads to troubles in the shore regions. Warm, calm weather with some rain and a lack of sunshine furthers the dying off and rotting away of the *Cladophora* and leads to the disagreeable condition mentioned.

Since the main masses of green thread algae die generally in July and August, there was formerly a period in autumn when there were comparatively few shore algae. But 1961 brought a new surprise to Lake Zürich. In September and October considerable quantities of *Hydrodictyon reticulatum* developed in various places on the shore. The network of this algae that at first floats on the water is later thrown onto the dry beaches by the waves and forms an unsightly line at the water level. Masses of plankton algae and shore algae also have a directly detrimental effect, but even worse are the effects when they die off in the lake. The most serious is the resulting lack of oxygen just above the bed of the lake in the metalimnion (possible also in the whole hypolimnion) and in the surface water near the shore. The nitrogen and phosphorus combinations set free from the dead algae return to the cycle again and promote the development of more algae. An important conclusion is drawn from this knowledge: only if it is possible to reduce considerably the superabundant production of plankton and shore algae in a eutrophic lake can a permanent improvement in the state of the lake be achieved.

B. Conditions for the permanent improvement in the state of rivers

Contrary to circumstances in lakes, the mechanical/biological purification of sewage discharged into rivers usually remedies the lack of oxygen - at least where the current is strong enough. In rivers with abundant nitrates and phosphates, algae and higher aquatic plants develop rapidly. Where the current is strong, the problems created by such algae are the least serious. In the stretches of river that are naturally slow flowing, however, mainly higher aquatic plants develop. For fishing, a thick growth of weeds leads to deterioration and affects the sport. The flow of water is also checked, which leads to an increase in the average water level. Swimming is impossible in such rivers. A vigorous proliferation of algae and aquatic plants lines the bed, so to speak, with a fur of organic material. The surface of the fur may have fresh water running over it continuously so that it offers animals favorable living conditions. On the underside of the fur, however, decomposition processes are already taking place and a layer lacking oxygen is developing. This applies especially to species between stones as well as in stagnant corners and holes in the stream bed and also in the cavities made by fish for spawning. If the spawn is covered over with mud, the eggs will be destroyed by fungi and bacteria.

The silting of the river bed is also disadvantageous to the condition of ground water used for drinking water or for commercial purposes. Replenishment of the ground water supply is made more difficult, and the water draining from a river choked with mud has less oxygen than an oligotrophic river does.

Plant nutrients are especially undesirable in rivers where the flow is used for producing electricity. Where the water flows into a pressure main, the original river bed has very little water, so the force of the current is less and allows an especially striking development of undesirable algae. If an attempt is made to maintain the original water level by damming, the growth of weed and algae occurs particularly where the depth of the water is less than 2 meters. If water from a river rich in nutrients is used for producing electricity, there should be an adequate quantity of water (residual water) in the original river bed - not only to preserve the natural beauty but also to protect the river itself. The mechanical and biological purification of sewage discharged into lakes and rivers cannot, in either case, be regarded as a permanent measure to improve their state unless the minimum nutrient substance is removed from the sewage.

V. Means of permanently improving the state of lakes and rivers

A. The permanent improvement of the state of lakes

1. Prevention of sewage discharge

There are lakes where the eutrophic condition is wholly or mainly caused by the discharge of sewage from the communities on their shores. The surest way of improving the condition of such lakes is to collect the sewage of all the communities in large pipes, purify it, and discharge it into the lake at its outlet. As a rule, such a system of drainage will have to surround the lake (circular drainage system).

On Lake Zürich, the sewage formerly discharged into the lake, from part of the town itself and also from the communities of Kilchberg and Zollikon (a total of over 50,000 inhabitants), for the past 50 years has been collected into drains and discharged into the lake at its outlet. Actual circular drainage systems to conduct sewage to the outlet of lakes have been planned in various places, e.g., the Lake of Hallwil in Switzerland and the Lakes of Tegern and Schlier in Bavaria. Since circular drainage for sewage generally requires more pumping, the running costs are considerably increased. Lohr (1961) reckoned with an annual expenditure of DM 18 (4 dollars) per inhabitant. Where there is not a great deal of water at the outlet of the lake, thorough purification is of special importance; otherwise new difficulties will arise. In large lakes with long and irregular shore lines and in lakes where the outlets are already rich in nutrients, the effective discharge of the nutrients into the lake outlet is difficult. In the following comments, therefore, other possibilities of combating eutrophication of lakes are discussed.

2. Nutrients removal from sewage

The removal of all the salts regarded as fertilizers cannot be dealt with here. Generally speaking, it is sufficient to remove the minimum substances, or the purification of the sewage can be such that a definite and important nutritive substance becomes a minimum substance in the lake. In my opinion, in the test areas known to me, phosphate is most suitable for removal for the following reasons (Thomas, 1949, 1956/57):

1. Phosphate is present only in small quantities in oligotrophic lakes in the Zürich area;
2. the natural tributaries running into these lakes contain very little phosphate as long as they are not subjected to human pollution, but contain large quantities of nitrate;
3. rain water often contains large quantities of nitrogen compounds that can be utilized by plants;
4. bacteria and blue algae living in the water are, under certain conditions, able to combine gaseous nitrogen organically;
5. a high percentage of the nitrogen compounds from the putrefying parts of organisms return to the cycle of substances as phosphorus compounds;
6. phosphates can be more readily eliminated from sewage than nitrogen compounds (for tests concerning the elimination of nitrogen from sewage cf. Bringmann, 1961).

One recommended method of eliminating phosphates from sewage is with aluminum sulphate (Lea, Rohlich and Katz, 1954). Another method is with iron chloride (ferric chloride) or a mixture of iron chloride and iron sulphate (Thomas, 1955b). In the mechanical/biological purifying plant on Lake Greifensee, Switzerland, 80 to 90 percent of the phosphates have been eliminated with ferric chloride (partly mixed with ferric sulphate) for the last 3 years. At present the plant purifies the sewage from about a 20,000 population. Since some communities are still discharging unpurified sewage into the lake, the condition of the lake is not yet permanently improved.

If the sewage from an industrial plant is very rich in phosphates, it should be utilized for agriculture or precipitated within the plant. The higher the phosphate content of the sewage, the more expensive it is for the community to purify it. If 80 to 90 percent of the phosphates is precipitated in a purifying plant during the whole year, the additional cost in the Canton of Zürich amounts to about 50 cents to a dollar per head, if the process developed by the author is used (a saving of about 50 percent).

3. Intensive development of fishing

As long as the conversion from organic substance into fish meat is possible in a lake, advantageous use is made of the fertilizers. Removing fish from lakes means a step towards preventing eutrophication (Thomas, 1944).

4. Algae and higher plant removal

Where green thread algae *(Cladophora, Rhizoclonium,* etc.) or higher aquatic plants *(Potamogeton,* etc.) profilferate abundantly on the shores, as many of these plants as possible should be pulled out by boats and then destroyed on the shore. This not only improves conditions in the lake but also contributes to the prevention of eutrophication because decaying algae in the lake release fertilizers.

5. Chemical intervention in lakes

Tests on *Cladophora* and *Rhizoclonium* cultures showed that these algae are very sensitive to zinc and copper salts (Thomas, 1962). I have never heard of combating algae on lake shores with zinc salts. Copper sulphate is used (e.g., near bathing beaches on the Lake of Zürich) to destroy the water snail *Limnaea auricularia* L., which is attacked by trematodes and annoys swimmers by releasing Cercarias.

Algae poisons might possibly be used successfully at individual places on the lake shore; however, if the algae are not attacked all round the shores of the lake, the wind can still blow algae growing in one place to any other point. Toxic substances used to control algae can also damage all other shore organisms as well as humans, especially where the water is used for drinking purposes.

6. Aeration of deep water in lakes

Attempts to aerate deep water in lakes have already been made in both America and Europe. Extensive tests have been made in Lake Pfäffikon, Switzerland (results not yet published). Air blown into the deep water is brought upwards by the rising air bubbles and becomes partly mixed with the surface water. During the summer, the epilimnion thereby becomes colder and the deep water warmer. Since troublesome fertilizers remain in the lake, however, the mass development of algae begins again and when it decays there is a sudden decrease in the oxygen content. The artificial warming of the deep water achieved in this way is undesirable for drinking water supplies. The unstable conditions produced also endanger fish life.

7. Drawing off deep water

Pumping or siphoning off deep water to the outlet of the lake during the summer period of stagnation removes part of the excess fertilizers present in the hypolimnion. During this process, water lacking oxygen is replaced by that rich in oxygen. The layer of warm surface water that has plenty of oxygen can be somewhat increased by drawing off the deep water - a process that is easy to regulate and control. (Thomas, 1944, p.194; 1948, p.175; 1956/57, p.207). Olszewski (1961) gives a good example of the practical application of this method. To avoid harm to the epilimnion by secondary oxygen consumption, the layer drawn off in the first year should not be too deep.

Drawing off deep water is the most successful method of artificial intervention in lake conditions.

8. Transferring fresh water to lake depths where the oxygen content is low

This treatment produces results similar to those achieved by blowing air into the deeper parts of lakes. There is no decrease in the fertilizers present in the lake so no decrease is expected in the growth of algae. In my opinion, this method is recommended only in special cases and not as a general means of improving the condition of lakes.

B. Permanent improvement in the state of rivers

If all sewage going into a river is mechanically and biologically purified, the condition of the water and degree of purity can be considerably improved. But, as has already been mentioned, there will still be troubles and damages caused by plant nutrients. Should we also eliminate in our rivers the proliferation of plants caused by nutrients? Most people concerned with the eutrophication of rivers - whether they are experts on the subject of water or outside observers - expect the proliferation of photosynthetic organisms in rivers to be extensively prevented by measures for preserving the purity of natural stretches of water. Taking this into consideration I believe that we should spare no efforts to prevent proliferation of aquatic plants and algae in rivers. What means have we of accomplishing this?

1. Protection of water-tapping areas and places used for swimming

Water-tapping areas or swimming areas near inhabited places can be protected by discharging sewage into the river below these points. This, however, means that the improvement is only local.

2. Elimination of phosphates from sewage

It has already been mentioned that phosphate is the most suitable for removal of any of the fertlizers as far as lakes are concerned. For the same reasons it is also recommended that phosphates be eliminated as far as possible from sewage discharged into rivers. It is hoped that this will reduce the growth of algae and aquatic plants.

3. Intensive development of rivers for fishing

The development of rivers for fishing, at least in Europe, is already under way. It is hardly to be expected that an increase in river fishing would result in any appreciable decrease in the growth of plants.

4. Removal of algae and higher plants

In rivers very much overgrown with aquatic plants it is necessary, at least locally, to cut out the plants and remove them from time to time. This is difficult and expensive but it has immediate results. Without this measure, the decaying plants in the deeper parts of the river cause damage.

5. Chemical intervention in rivers

In Europe there has been little experience of reducing plant growth in rivers by poison. In small streams in Zürich used for breeding trout, green thread algae were eliminated with Dowpon, without harm to the fish.

SUMMARY

A plentiful supply of fertilizers reaches algae and higher aquatic plants in rivers and lakes by means of sewage - even when this is mechanically and biologically purified. Nitrates and phosphates, which are particularly important as fertilizers, stimulate the growth of algae and higher aquatic plants, which leads to manifold damages and complications. A direct measure against the eutrophication of rivers and lakes that promises great success is the thorough removal of phosphates from the sewage. Where considerable quantities of phosphates reach the sewage from industrial areas they should, where possible, be removed from the sewage within the plants, or the sewage that is rich in phosphates should be used for agricultural purposes. Reference is made to some further aids to prevent the eutrophication of lakes and rivers. The special limnological character of each type of sewage must be considered when choosing preventive measures.

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THE BIOLOGICAL REMOVAL OF NITROGENOUS COMPOUNDS FROM SEWAGE TREATMENT PLANT EFFLUENTS

*William M. Beck, Jr.**

CONDUCT OF THE STUDY

From the previous talk it should be obvious that the increasing nutrient content of our inland waters is becoming a major problem. That sewage treatment plant effluents are in part responsible is unquestioned.

In the summer of 1959 a pilot plant study was organized at the Orlando, Florida, sewage treatment plant for the purpose of evaluating methods of removing nutrient compounds biologically. For this study a two-section sludge-frying bed was covered with a thin layer of well-digested sludge to stop seepage. A metered portion of the final plant effluent, prior to chlorination, was diverted to this bed.

The Orlando plant is well designed and skillfully operated. Treatment consists of primary clarification, sludge digestion, two-stage trickling filtration, and secondary clarification. The final effluent, prior to chlorination, had an average biochemical oxygen demand of 8 milligrams per liter.

During July 1959, three weeks were devoted to a study of biological removal of nutrients in this pond. Producers consisted of an almost pure culture of *Chlamydomonas* sp. Because of our active changing of retention time in the pond no attempt was made to measure the quantities of algae. Consumers consisted of *Chironomus decorus* and *C. fulvipilus* (34,992 per square meter, of which an estimated 95% were the former species and 5% the latter). These figures are based on a single sample (0.5 ft ^{2}).

Retention time was either increased or decreased every few days to obtain data about the relationship between loading and biological removal of nutrient elements. It is realized that such frequent changing did not necessarily permit a full biotic response to the altered conditions; however, results show that such response did occur.

Analytical determinations were made by Mrs. Foymie S. Kelso and Mr. Marvin L. Wicker, Florida State Board of Health. My own work consisted of chemical and biological sampling, and determination of dissolved oxygen, pH, and temperature during the night sampling. "Standard Methods" was followed in chemical determinations.

It is a pleasure to acknowledge the complete cooperation and generous assistance of Mr. J. Howard Worsham, Superintendent, Orlando Sewage Treatment Plant, and of his associates.

Samples were obtained hourly from the pond influent and effluent. Dissolved oxygen, pH, and temperature were determined immediately and nutrient analyses were made on 24-hour composites. Nitrate, nitrite, and ammonia analyses were made separately and the results combined and reported as total nitrogen. Both orthophosphate and total phosphate analyses were made; these results were not reported because of poor efficiency of biological removal. Effluent samples were filtered through paper with a low vacuum to remove algae.

RESULTS

Table 1. TOTAL NITROGEN REMOVAL

Date (July)	Influent, mg/liter	Effluent, mg/liter	Removal, %	Gal/day	Retention, days
8	11.5	4.9	57.4	70,000	1.12
9	13.4	1.4	89.6	33,000	2.38
10	13.0	1.8	86.2	29,000	2.71
13	14.4	10.1	29.9	111,000	0.71
14	14.8	9.1	38.5	111,000	0.71
15	14.0	9.3	33.6	70,000	1.12
16	12.9	6.5	49.6	76,000	1.03
17	14.0	7.6	45.7	77,000	1.02
20	13.4	7.8	41.8	77,000	1.02
21	15.1	5.2	65.6	40,000	1.96
22	12.3	2.5	79.7	34,000	2.40
23	14.8	2.2	85.1	40,000	1.96
Maximum		15.1	10.1	89.6	
Mean		13.6	5.7	58.6	
Minimum		11.5	1.4	29.9	

Table 2. CHEMICAL AND PHYSICAL FACTORS

	O_2, mg/liter	pH	°C
Maximum	35.7 [a]	10.6	34.5
Minimum	0.0	6.7	26.0

[a] At 34.4°C = 496% saturation.

DISCUSSION

As will be seen from Table 1, removal of total nitrogen ranges from a minimum of 30 to a maximum of 90 percent. Figure 1 shows the relationship between retention time and percentage removal. Maximum nitrogen removal occurs with 2 to 2.5 days' retention. Total phosphate removal is much less efficient, although there is a direct relationship between phosphate removal and retention time. As in the case of nitrogen, phosphate removal varied directly with retention time.

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* Florida State Board of Health

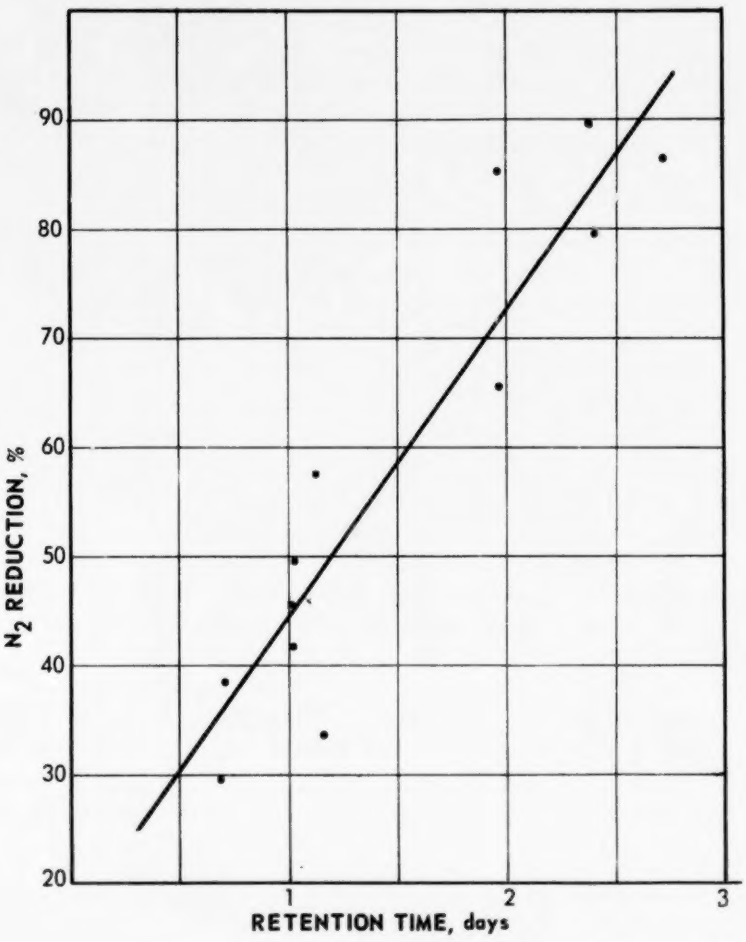

Figure 1. Relationship between retention time and percentage of total nitrogen removal.

OBSERVATIONS

Figure 2 presents the diurnal cycle for dissolved oxygen and pH. The oxygen curve is especially interesting. Following a minimum concentration of dissolved oxygen just before dawn there is a rapid increase, with supersaturation occurring by 0900 hours. The peak was reached at 1500 hours. The rapid drop after 1500 hours represents a phenomenon that occurred several times during the study. The entire pond, without warning, starts to effervesce. This results in oxygen flotation of virtually the entire crop of algae. The algae may then be removed by skimming. As noted in Figure 2, this effervescence resulted in an excessive loss in oxygen and a subsequent increase in dissolved oxygen prior to sunset. This was due to agitation by a thundershower. On no other day did this occur. The shower did not put the algae back into suspension.

To support our contention that the algae crop harvested itself by oxygen flotation, we decided to attempt agitation of the pond just prior to the oxygen peak at 1500 hours. We did not want to disturb the layer of sludge on the bottom of the pond. Finally we resorted to the use of an old-fashioned, vibrator-type automobile horn and a storage battery. The horn, wrapped in thin polyethylene and immersed in the pond, was honked briefly, and produced effervescence and algae rise in a small area.

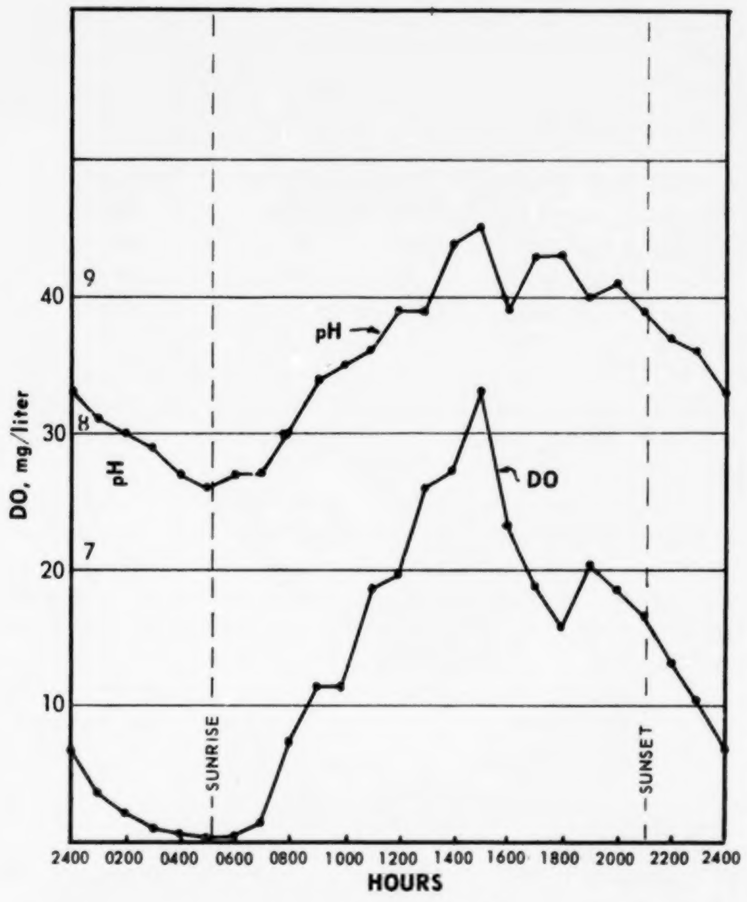

Figure 2. The diurnal cycle for dissolved oxygen and pH, July 14 to 15.

CONCLUSIONS

1. Removal of total nitrogen by the methods reported here reaches a high level under properly controlled conditions.

2. More efficient methods of removal of phosphates are needed.

3. The results obtained in this project warrant more careful study.

DISCUSSION

The greater part of the discussion dealt with methods of removing phosphates from waters. A three-stage process was described in which there is primary clarification followed by the addition of ferric chloride and aeration to precipitate the phosphates. The stage is followed by settling. One discusser suggested that the addition of ferric chloride at the middle stage would be uneconomical because of the adsorption of iron and suggested that the addition of sand at this stage would be better. It was stated, however, that the addition of sand would result in too much sludge, and, therefore, would be uneconomical from that standpoint.

A report was made that Sweden has been experimenting with a flotation method of removing phosphates by employing supersaturated water under pressure. This method provides for over 90 percent phosphate removal, and if a flocculent is added, better results are realized. Other experimental work was discussed in which water hypacinth was used to remove phosphates and nitrogen. Little success was realized, however.

In discussing the eutrophication of lakes, a statement was made that if the phosphate levels can be maintained below 20 ppb, algal blooms will not ordinarily be encountered. The chemistry of each body of water, however, must be considered separately. The point was also made that phosphate removal alone cannot guarantee elimination of algal blooms, but nitrogen must also be considered, especially since some of the algae are capable of fixing atmospheric nitrogen. Some success is being realized in eliminating eutrophication in Florida lakes with aeration.

The Artificial Eutrophication of Our Waters -
Discussion

An inquiry was made regarding the definition of an algal bloom. Some felt that the algal growth must cover the whole lake and not just part of it to be considered a bloom. Mention was made of the Great Lakes, however, where algal growths cause considerable problems, and yet, according to this definition, would not be considered blooms.

Mention was made that temperature is the primary reason for the seasonal occurrence of blue-green blooms and that blue-green algae are not part of the aquatic food cycle because of their apparent indigestibility.

DETERMINATION OF SAFE LEVELS OF TOXICANTS AND OTHER POLLUTANTS IN THE AQUATIC ENVIRONMENT

L. L. Smith, Jr., D. F. Alderdice, F. J. Trembley, P. C. G. Isaac, R. C. Pendleton, and C. G. Wilber, Chairmen

APPARATUS FOR BIOASSAY OF WOOD FIBERS WITH FISH EGGS AND FRY

Lloyd L. Smith and Robert Kramer *

No satisfactory apparatus for bioassay of suspended fibrous materials originating in paper-mill wastes was available for use with fish eggs and young fish. Equipment suitable for testing dissolved materials is inadequate for suspended wood fibers in varying concentrations. In order to determine the effects of wood fibers on eggs the material must be kept in suspension, the eggs must be agitated, and a continuous water flow past the eggs must be maintained. For use with young fish, material must be kept in suspension and a constant water quality maintained.

Fibrous materials in paper mill effluents vary from a few microns to 5 millimeters in length and their diameters vary with the tree species and the degree of separation of the wood. The physical character of the material varies with the chemical treatment to which it has been subjected. Wet wood fibers are slightly heavier than water but lighter than fish eggs usually deposited on the bottom.

TESTS WITH FISH EGGS

Two types of apparatus were used to test the influence of suspended fiber on the survival and hatching of fish eggs, (1) a continuous-flow system and (2) a closed system.

Continuous-flow system:-A continuous-flow system is desirable for tests with fish eggs because it removes metabolites, facilitates egg movement, and permits maintenance of constant water quality. The apparatus developed (Figs. 1 and 2) to achieve constant conditions delivers a measured flow of a standardized suspension of fiber to a mixing chamber where it is diluted to the desired concentration with fresh water of constant temperature and oxygen content. The standard stock suspension is pumped from the reservoir to the mixing chamber by a Sigmamotor peristaltic-type pump driven through a speed changer that permits accurate calibration of flow. The pump manipulates Tygon plastic tubes which run from the concentrated-fiber reservoir to the mixing chambers without joints or constrictions. Tubes of four diameters may be used simultaneously to provide four rates of flow to each of four separated mixing chambers. Two fibers at two levels or one fiber at four levels can be supplied to the test chambers. Liquid level in the mixing chamber is maintained with a float valve on the water supply. Outflow from the mixing chamber through the test chambers is regulated by raising or lowering the level of the overflows on the test chambers. The flow is adjusted to achieve the proper action among the eggs and a satisfactory suspension of fibers. By adjusting the density of the fiber concentrate, speed of the pump, and rate of flow any desired level of fiber load may be circulated through the test chambers. Constant agitation in the concentrate reservoirs and mixing chambers is required.

It has been practical to operate the equipment up to 26 hours on a single charging of the concentrate reservoirs, and the apparatus has been continuously run for 20-day periods. Test chambers are standard 1000-ml., 3-necked, distillation flasks. This equipment has the advantages of easy sampling of eggs through the open overflows during the run and is free from clogging in the metering pump. Oxygen in the water can be maintained at predetermined levels by regulating the oxygen content of water supply with nitrogen stripping columns or a mechanical degasser.

Closed system:—A closed system for tests of fiber with eggs was made by attaching aspirating tubes to a distillation flask (Fig. 3). Fiber and eggs were placed in the flask. The fiber suspension was then circulated at a rate which gave adequate agitation to the eggs. In runs exceeding 12 hours water and

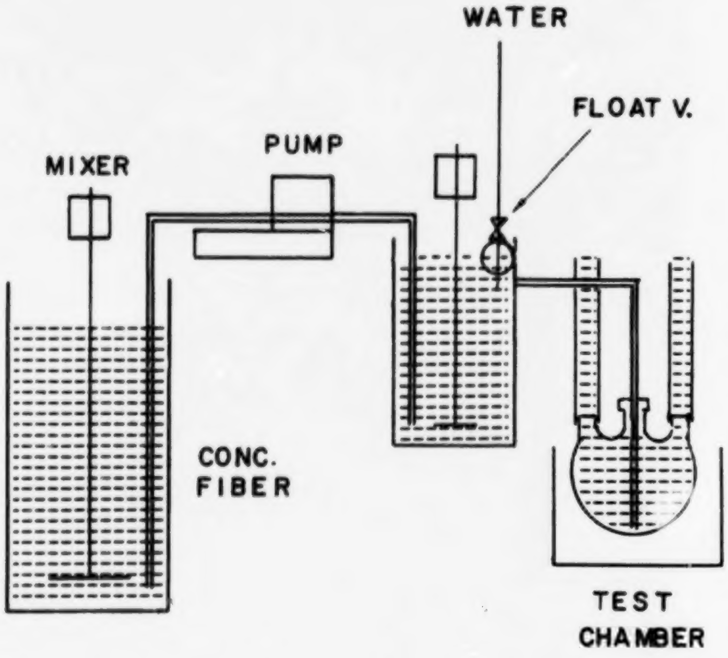

Figure 1. Schematic drawing of continuous-flow system for subjecting fish eggs to constant wood fiber loads.

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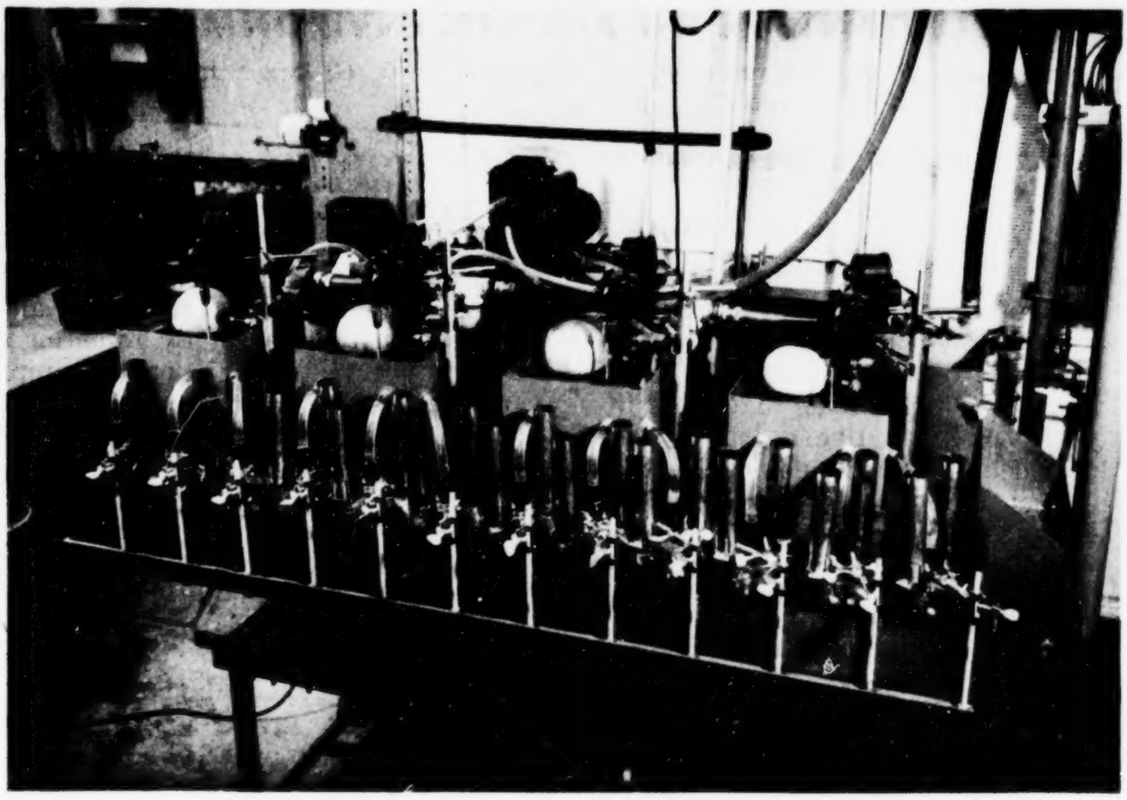

Figure 2. Continuous-flow apparatus for testing fiber loads on fish eggs.

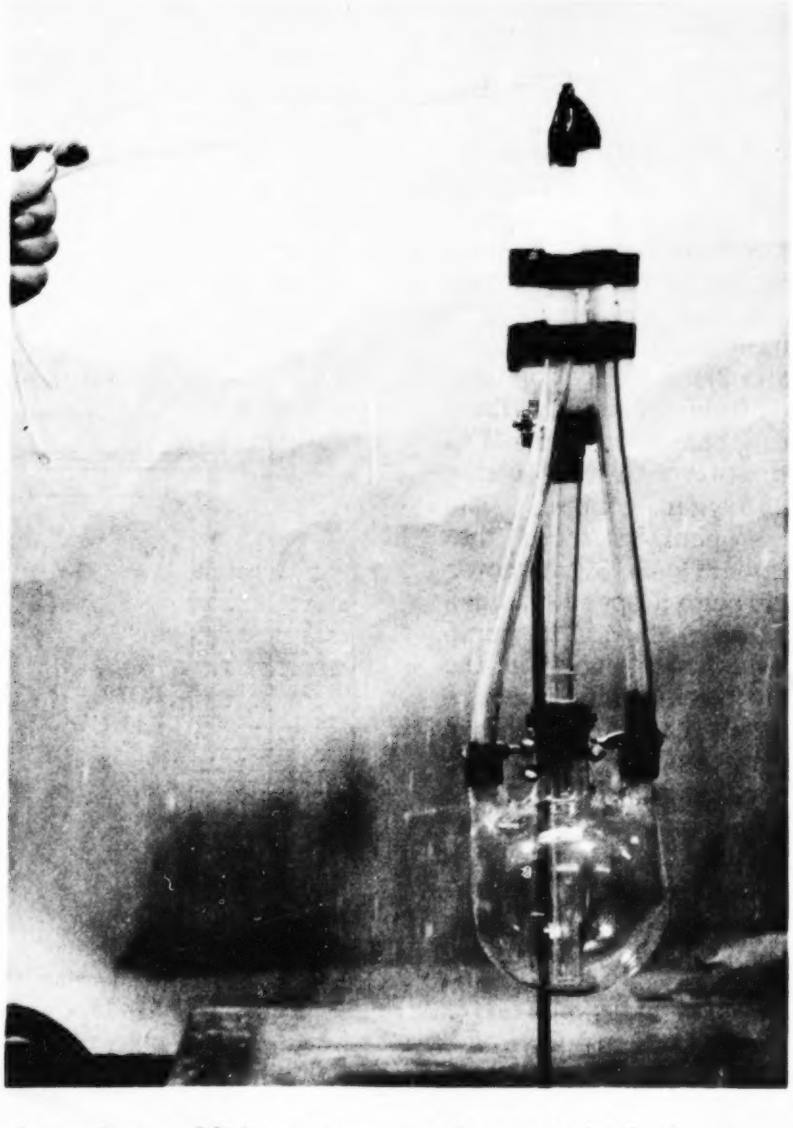

Figure 3. Closed water system for incubation of fish eggs in water with varying fiber loads. Water and fiber rises through outside tubes to upper reservoir and descends through central tube. Discharge of central tube at bottom agitates eggs.

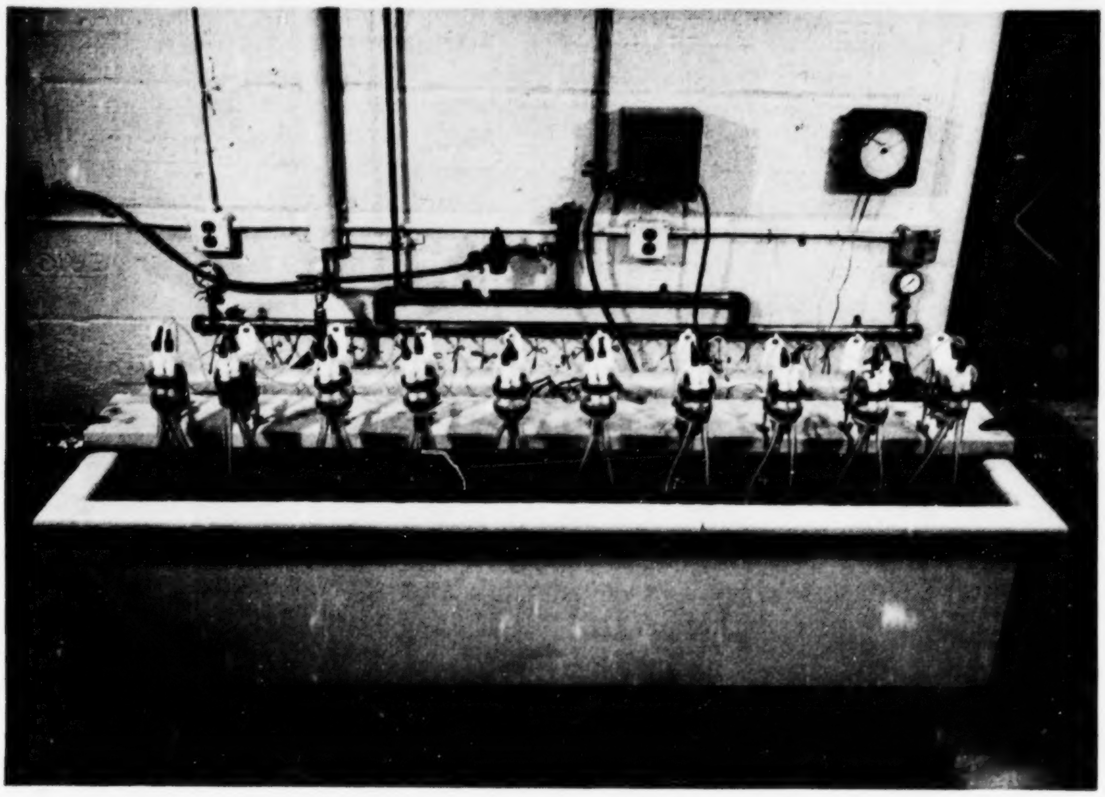

Figure 4. Battery of closed-system vessels immersed in water bath.

fiber were replaced every 12 hours. Constant temperature was maintained by immersing the battery of units in a water bath (Fig. 4). Uniform water flow in the different units was maintained by use of constant air manifold pressure. Flow induced by aspirators was varied by changing manifold pressure.

This system has the advantage of retaining fry after hatching and does not require continual preparation of large batches of standardized fiber concentrate. By adding nitrogen in proper proportion to air constant, predetermined oxygen levels can be maintained.

TEST VESSELS FOR FISH

A closed-water system employing glass hatchery jars 18 by 8 inches was found to be satisfactory to

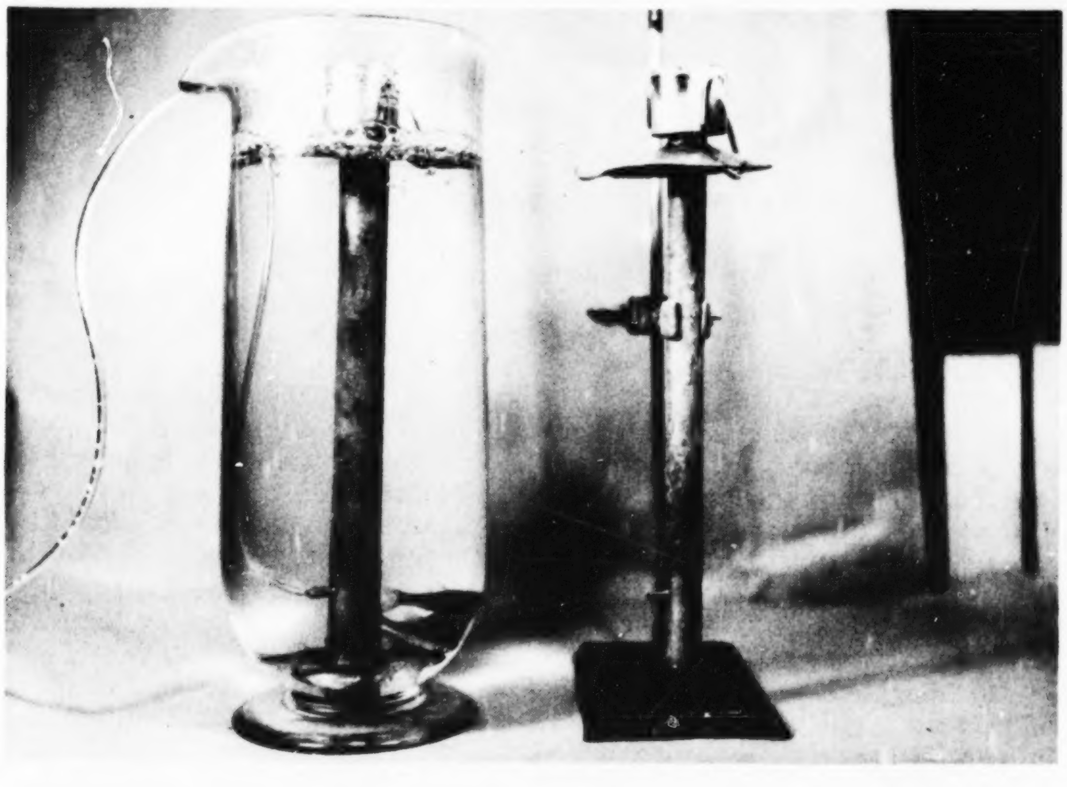

Figure 5. Closed-water system for subjecting small fish to various fiber suspensions.

test the effects of fiber suspensions on fish up to 5 inches in length (Fig. 5). Fish were placed in the jars with the water suspension of fibers. A gentle circulation of water was maintained by use of a central aspirator tube. Rate of circulation was controlled by adjusting the air pressure to the tube. A battery of 27 jars with varying treatments was operated simultaneously from a common air manifold. All jars were immersed in a water bath (Fig. 6) and held at a predetermined temperature in semi-darkness. Placing the apparatus in reduced light caused the fish to distribute themselves throughout the jars. Tests were run for periods up to 96 hours. Saturated oxygen levels were maintained but control of oxygen could be achieved by mixing air fed to the aspirators with appropriate volumes of nitrogen.

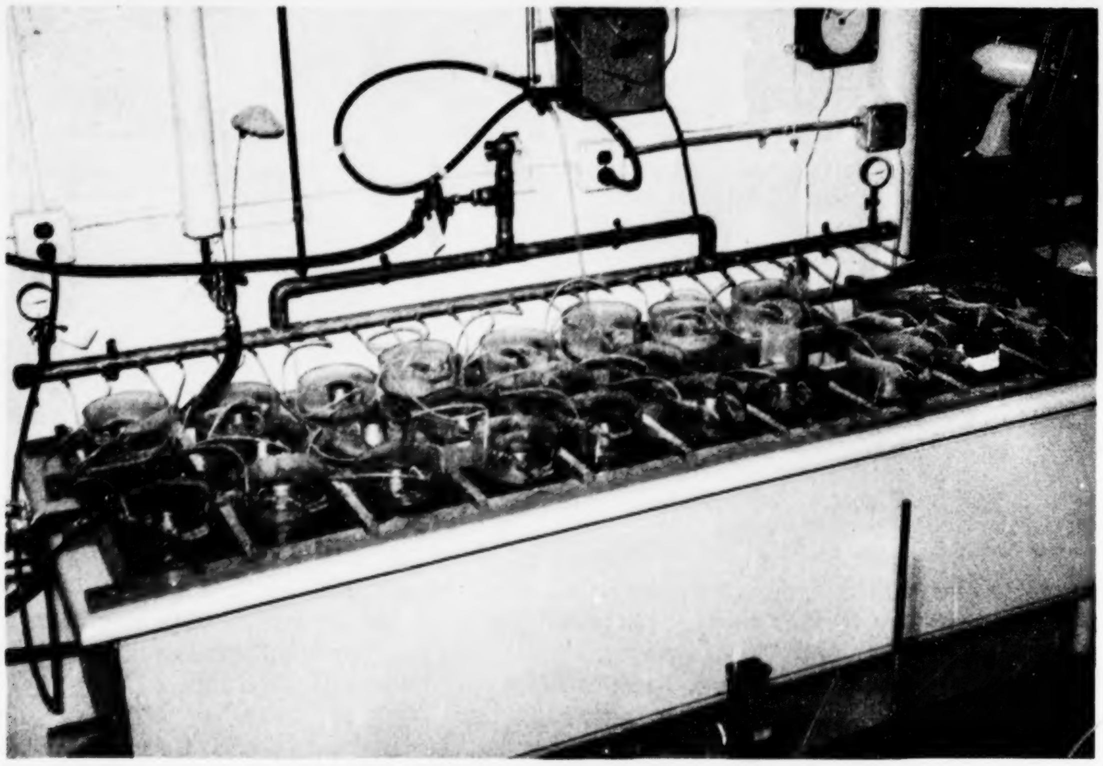

Figure 6. Battery of closed-system vessels for small fish.

THE DESIGN OF A NEW FISH RESPIROMETER

J. R. Brett *

INTRODUCTION

Studies on the respiratory metabolism of young sockeye salmon have been conducted in a respirometer designed to impose exact velocities and temperatures, thereby permitting measures of the oxygen requirements for swimming at any speed up to and including fatigue levels. The aim has been to obtain precise, reproducible data on normal fish such that subsequent tests involving abnormal conditions could be expected to reveal the presence of metabolic stress separate from normal variability. Since the metabolic rate is dependent on or influenced by a variety of environmental factors including temperature, salinity, oxygen availability, pH, and acclimation history, as well as such characteristics as size, condition (exercise and diet), excitation state (internal secretions), diurnal rhythms, starvation (post-absorbtive period), sex, and age, it was necessary to pay close attention to each variable and to design apparatus to meet the requirements.

APPARATUS

GENERAL CONSIDERATIONS

The design provides for the recirculation of water in a closed tunnel (containing the fish) at any given velocity, up to maximum output of the pump. New water can be introduced independently for sampling or flushing, or a continuous flow of water can be steadily exchanged.

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The metabolic rate is determined by measuring the change in oxygen content at regular intervals, followed by flushing to maintain oxygen level, or by measuring the difference in oxygen level between incoming and outgoing water of known flow rate.

The fish is forced to swim against the current by use of a small electric charge (4 volts AC) on a wire screen. The fish cannot do less than the minimum work to swim at the prescribed velocity, provided no areas of reduced velocity exist; it may do more by being over-excited and swimming against the walls of the chamber or forward screen. Hence, repeated values must be obtained at each velocity, with attention being directed to the minimum values which represent the non-excited levels. By progessively increasing the velocity in 1-hour steps the relation between swimming speed and metabolic rate can be established, with the extremes describing the basal and active levels.

PARTICULAR FEATURES (SEE ILLUSTRATIONS)

Structure. The drive unit including pump is separately mounted from the tunnel section and physically linked through rubber joints, reducing vibration transfer. The tunnel is of plastic tubing (2" PVC) with fiberglass expansion and contraction cones leading to and from the plexiglas fish chamber (4-1/2" ID x 12"). Freedom to swim is provided while reducing the total volume of circulated water (4.5 gal) to a minimum, without undue frictional loss and heat production.

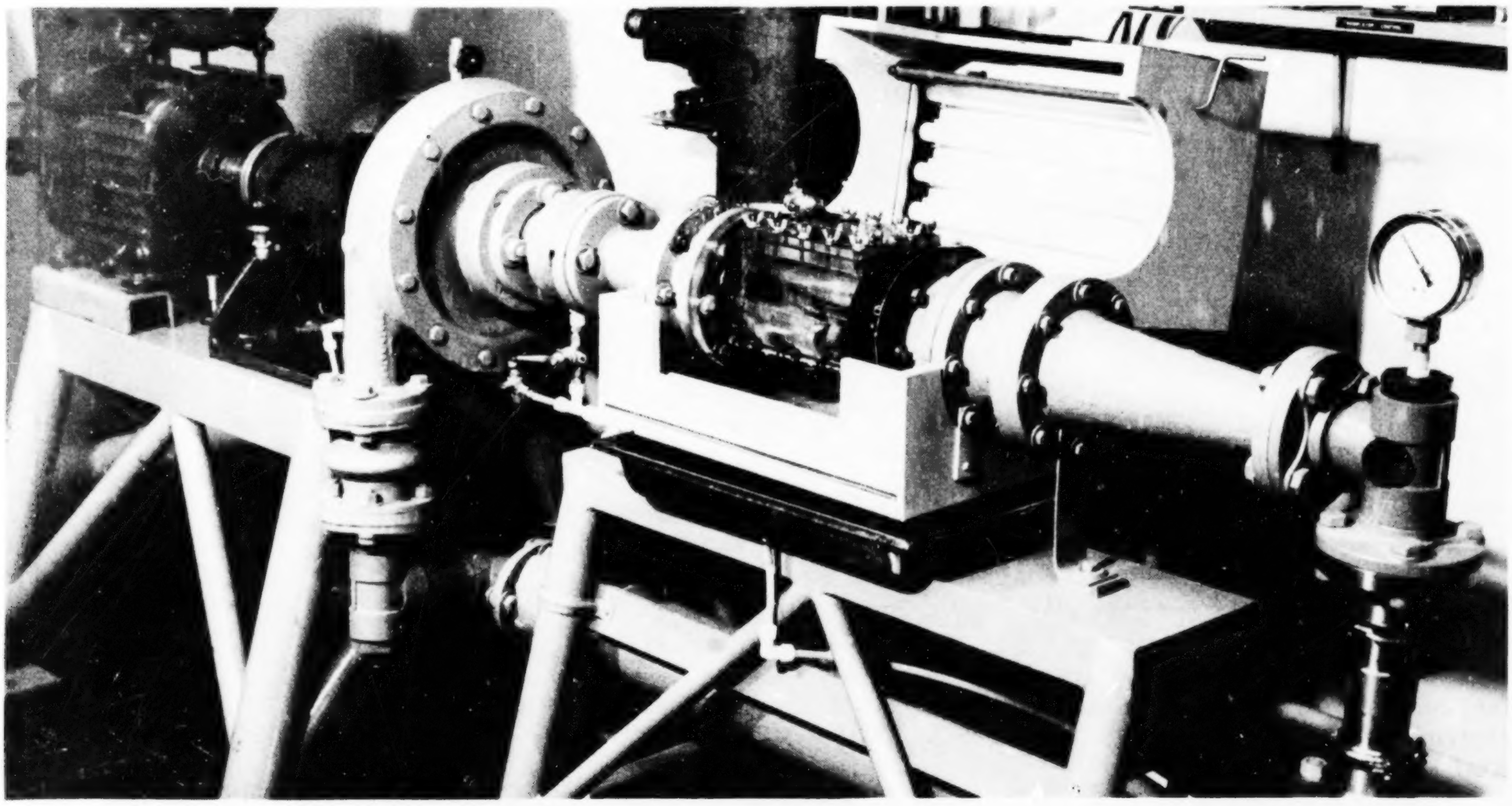
Drive Unit. A 3-HP electric motor top-mounted on a Carter Variable Speed Gear drives a centrifugal Worthington pump. Maximum output is approximately 450 gal/min against a developed head of 70 feet.

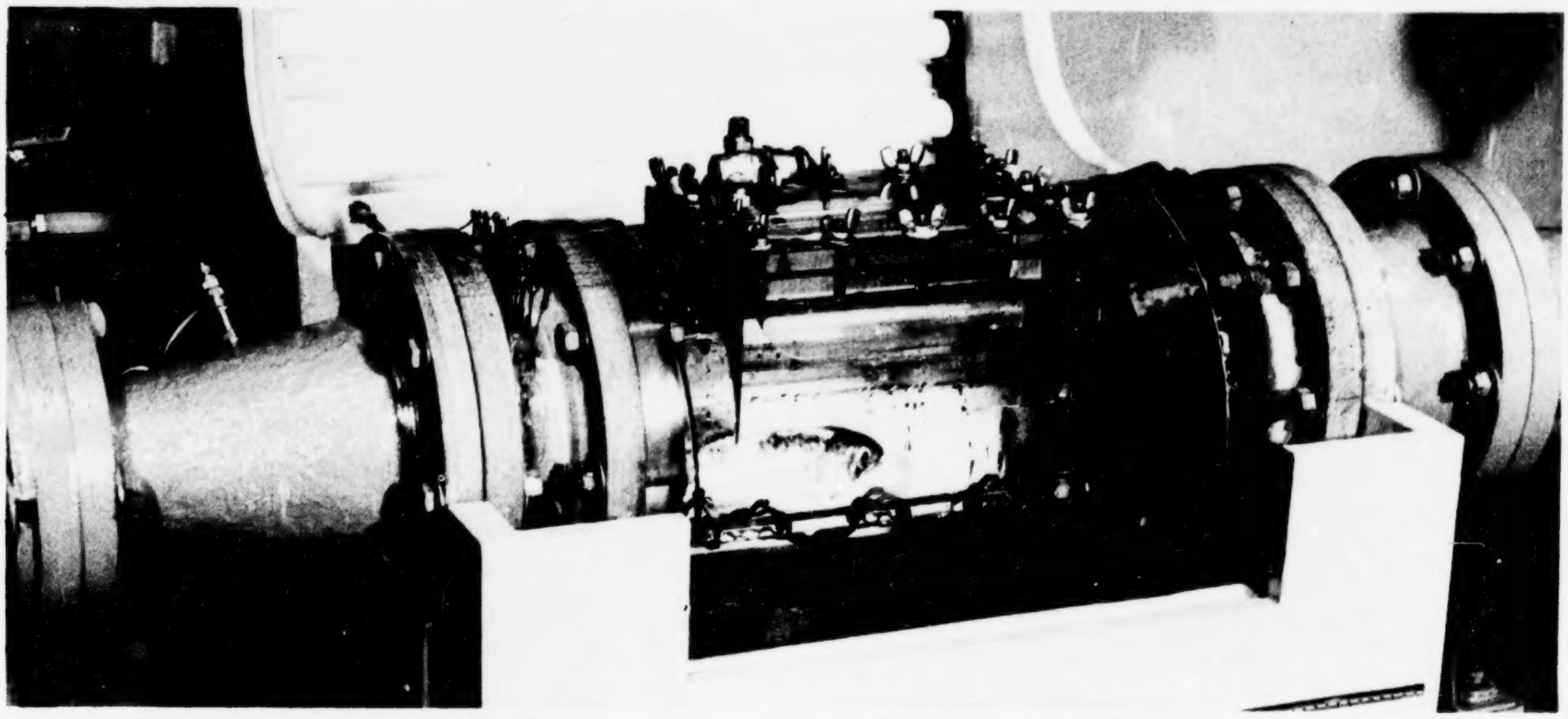
Velocity. A consistent, flat velocity profile occurs over the entire range of velocities (0-4 ft / sec) through the fish chamber. This is achieved by use of appropriately designed cones and a series of three, spaced turbulence grids preceding the chamber. Velocity is continuously measured by a Pottermeter bearingless, turbine-type flow meter calibrated for direct read-out for the rate through the chamber.

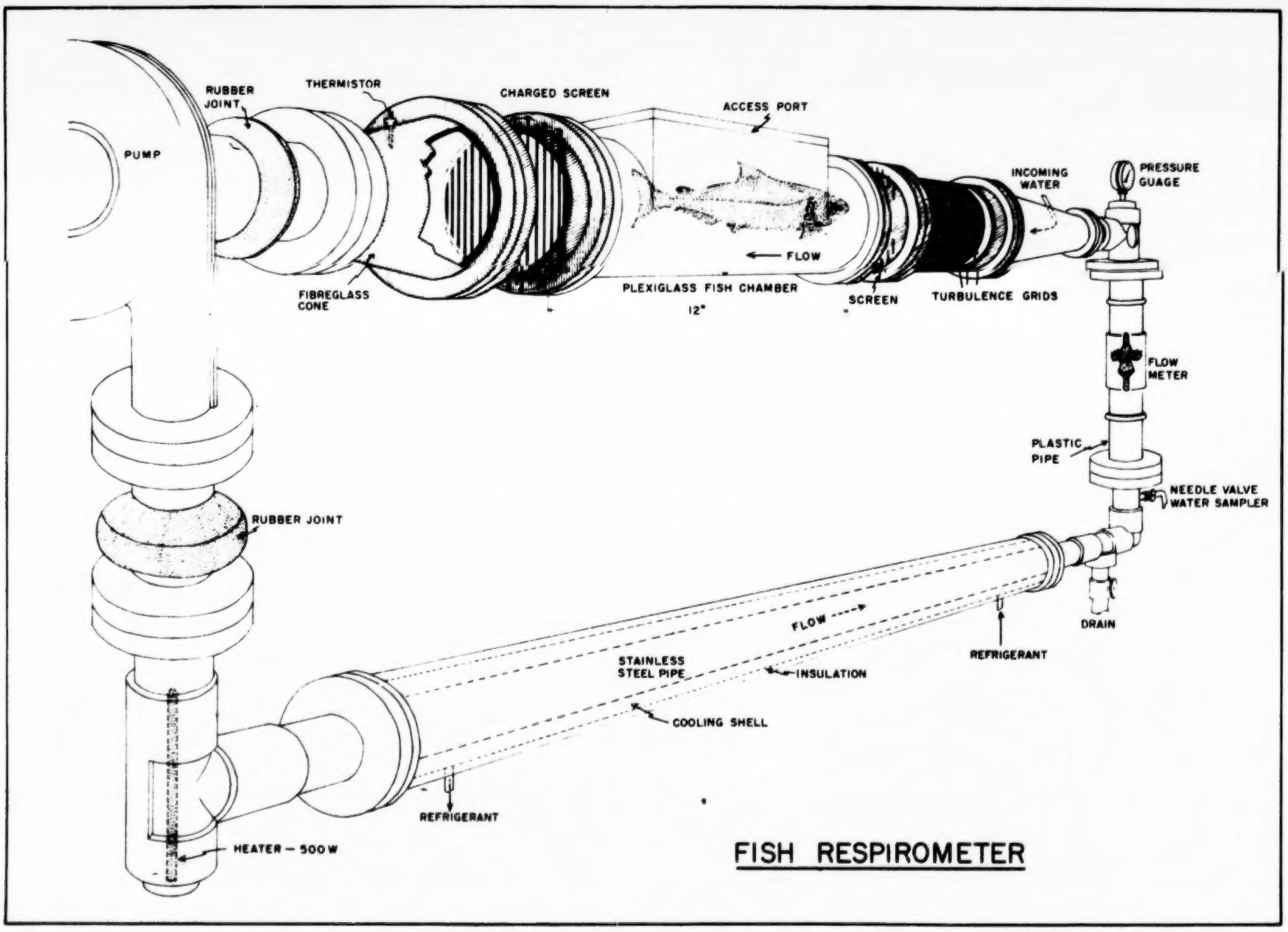
Temperature. A 500-W heater counteracts overcooling from a refrigerated heat exchanger. The latter is a stainless steel insulated cooling shell with glycol refrigerant (down to -15°C) recirculated from a separate cooling unit. Exchange water is thermally controlled prior to introduction.

Oxygen. Exchange or flushing water is presaturated to 105% of atmospheric oxygen and allowed to be respired down to 95% when the unit is on closed circuit. A needle valve water sampler controls rate of exchange during open circuit. Winkler titrations are employed but provision for an oxygen electrode is provided at the tee housing the pressure gauge.

Fish Chamber. A forward and back, electrified screen of piano wire limits movement over a 15-inch length. A black, forward cover-area is provided, and a contour-formed access port. Surrounding the chamber, and faced with insertable one-way glass, is a light-box with incandescent lumiline bulbs in series with a variable transformer. Very low lighting is used for resting studies. A black rubber mat contours the outside bottom third of the chamber to provide good reference and "bottom-relation" for the fish.






RUBBER JOINT
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PUMP
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PRESSURE GUAGE
FLOW
FIBREGLASS CONE
PLEXIGLASS FISH CHAMBER
12"
SCREEN
TURBULENCE GRIDS
FLOW METER
PLASTIC PIPE
NEEDLE VALVE WATER SAMPLER
RUBBER JOINT
DRAIN
FLOW
REFRIGERANT
STAINLESS STEEL PIPE
INSULATION
COOLING SHELL
REFRIGERANT
HEATER – 500W

FISH RESPIROMETER

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APPARATUS USED FOR STUDYING AVOIDANCE OF POLLUTANTS BY YOUNG ATLANTIC SALMON

John B. Sprague *

The essential part (Fig. 1) is a Plexiglas tube 134 cm (53 inches) long and 14.6 cm (5-3/4 inches) in diameter. Into each end of this tube peristaltic tubing-pumps feed 2 liters of water per minute via white plastic funnels. The water is drained from the center of the avoidance tube by four hoses leading to waste via a common chamber. The tube is kept a little more than half full, and is partially open along the top for insertion and removal of fish. Baffle plates of Plexiglas are installed 10 cm (4 inches) from each end. The other four lines which can be seen around the tube are merely crayon marks used to designate the position of the fish.

A third variable-speed peristaltic pump can add the pollutant to either side by way of the funnels. In the photograph, dye has been pumped into the right hand side.

The wooden doors close to shield the fish from visual disturbances. Above the avoidance tube can be seen Plexiglas heating chambers, gas equilibration columns, and constant-head containers. Underneath the table are stock-tanks of fish being acclimated.

The apparatus gave a sharp junction between "pure" and "polluted" waters. Best results were obtained by measuring the time spent in each end of the tube by actively swimming fish.

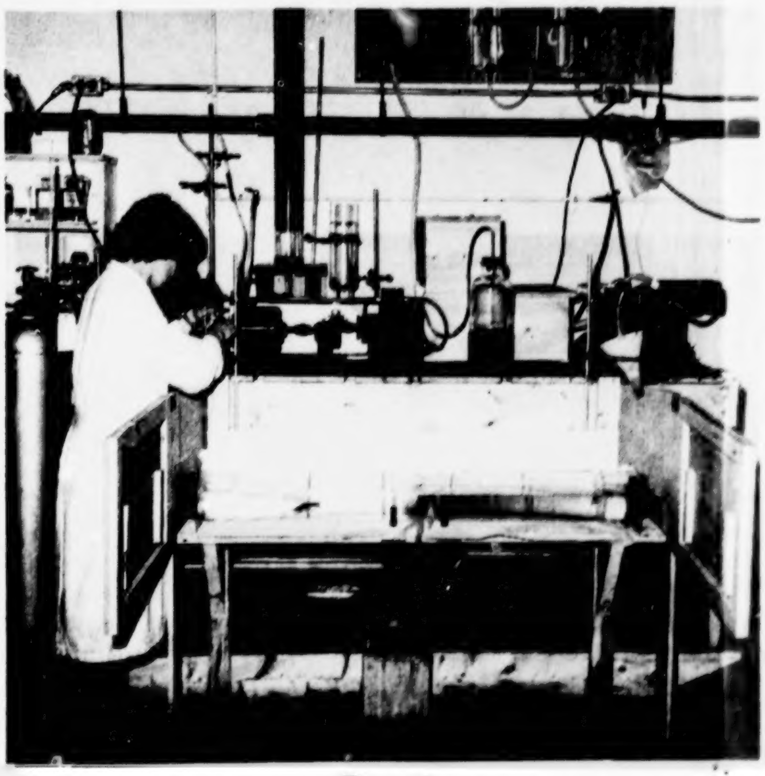

Figure 1.

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AN APPARATUS FOR TESTING THE SWIMMING HABITS OF FISHES

K. A. Pyefinch *

Observations on the leaping behavior of salmon and trout were carried out by T. A. Stuart in a channel 50 x 1.25 x 1.5 feet made of transparent perspex 1/4-inch thick. The channel was divided into two parts, the upper part 30 feet long and the lower 20 feet. The upper channel was mounted some 20 inches above the lower and connection between the two parts was provided by a perspex fish ladder. The long section of the channel could be tilted to below the gradient by means of an hydraulic jack. Provision was made for the insertion of dams and spillways of various sizes into the channels. These dams were also made from 1/4-inch perspex sheet and rectangular, triangular, and trapezoidal openings were cut into these dams.

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PHYSIOLOGICAL CONSIDERATIONS IN STUDIES OF THE ACTION OF POLLUTANTS ON AQUATIC ANIMALS

Paul O. Fromm *

During the past few years in our laboratory we have investigated the effect of hexavalent chromium (as chromate) on aquatic animals. Most of the results have been published and therefore it would seem redundant and superfluous to discuss these data in detail again. Rather, I would like to refer to these studies somewhat superficially while presenting my views on the need for a greater emphasis on a physiological approach in the study of some problems of water pollution.

Physiology, as you know, is that branch of biology that is primarily concerned with a study of life processes of living organisms or, stated differently, a study of the functions of organs and parts during life. There have been many studies dealing with the relationship between animals and their environment and, indeed, the ecological distribution of some species can be explained largely on the basis of the physiology of the animals involved. In addition, physiologists have also been concerned with the reactions of animals to changes in their environment--studies of the physiology of stress. "Stress," as used in this paper, may be defined as the conditions(s) resulting from any environmental change that disturbs the normal functioning of an animal to such an extent that its chances for survival are reduced. The pollution biologist, with a physiological approach and using fish as an assay animal, should be concerned with what occurs on the surface and within the fish in response to a pollutant; whereas, the physical chemist and limnologist should be concerned more with the physical and chemical characteristics of the water, and events occurring in the immediate environment of the fish.

At this juncture three questions might properly be asked: Why a physiological approach? Are present and past methods of investigation no longer acceptable? Is this just another "gimmick" to be used to obtain large-grant funds from various federal and state agencies? Answers to these questions may be gained by asking still another question: Is the study of non-acutely lethal concentrations of water pollutants really necessary? My answer to the latter is an unqualified yes!

In studies of water pollution perhaps the most widely used method for the evaluation of toxicity of materials to aquatic animals is the fish bioassay (TL_m determination) proposed some years ago (Doudoroff, et al., 1951). The mean tolerance limits serve only as useful indices of the relative toxicity of the substances tested and do not represent concentrations deemed safe or harmless. In a somewhat similar approach, Herbert (1961) in England, has presented data from short-term toxicity studies using a log-log plot of period of survival versus concentration. A threshold of toxic concentration is defined as that concentration that would kill no more than 5 percent of fish as susceptible as trout within 3 months. In this as well as the fish bioassay procedure, the extrapolation of values obtained to concentrations that are supposedly biologically safe is somewhat hazardous to say the least. This is illustrated by the effect of lead on fish.

In sufficiently high concentrations lead ions combine with mucus covering the gills and give the typical protein coagulation reaction that results in interference with the respiratory process. As a result death is rapid and is due to suffocation. Dawson (1935) has shown that after sufficient exposure (16 to 183 days) to very low concentrations, lead can act internally causing significant changes in the blood (pronounced secondary anemia), liver (increase in macrophages with progressive accumulation of pigment of erythrocytic origin), and spleen (decrease in erythropoietic activity and presence of increased numbers of pigment macrophages). A TL_m value for lead, determined over a period of not longer than 96 hours, involves the use of acutely lethal and non-acutely lethal concentrations to provide data on which the determination of a biologically safe concentration of the metal is based. The fact that the cause of death may be changed entirely when different concentrations of the metal are used is not taken into account in any way. The question with respect to short-term studies is, "How (if at all) are those fish that survive the test affected by the pollutant being studied?" The question is not answered by the fish bioassay technique, nor was it intended to be. Answers must be obtained from long-term (chronic) studies with low concentrations of pollutants. This does not mean to imply that the fish bioassay or other short-term studies have no value and should therefore be discarded; the author feels, however, that if real progress is to be made in determining biologically safe concentrations for various pollutants we must turn to the assessment of the effects on biological material of non-acutely lethal amounts of toxicants. With death ruled out as an endpoint, we are thus forced to establish procedures for investigating alterations of functions in living animals in response to environmental stress (pollution).

In assessing the response of aquatic animals to pollution the determination of one or a number of specific changes in the internal state of an animal may be made. Specific changes or differences resulting from pollution stress may be decreased liver glycogen, hormonal depletion, elevated or depressed blood-sugar levels, accumulation of lactic acid in the blood, or other chemical changes. The remark by Gertrude Stein to the effect that 'a difference, to be a difference, must make a difference' is particularly pertinent to this discussion. Changes that may be useful parameters in determining the severity of pollutional stress may be of a histopathological nature such as necrotic changes in various organs, cirrhosis of the liver, degenerative changes, and many others. Additional changes, which could be partially included in either of the above categories, are blood-vascular changes such as alteration of hematocrit, hemoglobin, plasma proteins, or changes in the amount and type of formed elements in the circulating blood.

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Some of the suggested parameters have been used in our laboratory. For example, it was found (Schiffman and Fromm, 1959) that trout exposed to various concentrations of hexavalent chromium responded by showing an increased hemoconcentration (increased hematocrit). This rise in hematocrit was accounted for by an increase in cell number, cell volume, and a probable decrease in plasma volume. It was suggested at the time that investigations should be carried out to determine whether the rise in hematocrit could be used as an index of chronic toxicity. Research with largemouth bass (Fromm and Schiffman, 1958) showed that chromium caused severe pathological changes in the gastrointestinal tract of exposed fish that in all probability completely destroyed its digestive function. In trout exposed to 1 ppm hexavalent chromium for up to 38 days the blood-sugar levels were high (90 mg%) after 20 days and dropped to about 40 mg% after 38 days' exposure, whereas the blood-sugar level in controls ranged from 40 to 60 mg%. Lactic acid showed a gradual rise during the period of exposure; however, no differences were noted between controls and chromium-exposed fish. Liver glycogen values were similar although quite variable in control and exposed fish with values ranging from 0.1 to 2.4 gm%. Muscle glycogen response was similarly variable and the values were significantly lower than those for liver glycogen, ranging from 0.01 to 0.37 gm%. Tissue respiration studies revealed no differences in the metabolism of slices of liver, kidney and pyloric caeca tissues of fish exposed to chromium when compared to similar tissues from control fish. There were differences between types of tissues, the average rate of oxygen consumption (Q_{O_2}) being 2.7, 2.0, and 1.6 microliters per milligram per hour for kidney, liver, and pyloric caeca, respectively, measured at 13° to 15°C. These data on the physiological responses of trout to exposure to a 1 ppm concentration of chromium are preliminary and few in number and have not heretofore been published. It can be seen in this attempt to give an overall clinical picture of the effect of chromium on fish that many of the parameters investigated showed no significant change in the exposed fish.

Various difficulties are encountered in collecting data reported in the previous paragraph. Three graduate students and the author were simultaneously involved in the collection of some of these data. In most cases one is confronted with a notable lack of good control data and must therefore spend considerable time establishing normal values. Even when data are available one is usually amazed at the tremendous range in the values reported as well as the number of different journals and assorted publications from which the information must be gleaned. This is especially true of reports dealing with fish; some attempts have been made, however, to survey the pertinent literature in this field (Hunn, 1960; Schwartz, 1961). In addition to literary barriers and such factors as variations in the nutritional status of experimental animals, sex differences, etc., more hindrances to productive research appear such as the necessity of drastically modifying methods originally designed for use with larger animals. Several new microtechniques (viz. microhematocrit method) have appeared in recent years, however, which should prove helpful in work with smaller vertebrates and invertebrates although the methods devised have been primarily for clinical procedures in human medicine.

Data of the type under discussion are usually obtained from research carried out in a laboratory or possibly at a fish hatchery. In certain cases histopathological information may be obtained from material obtained from aquatic animals taken from polluted waters. In any event, highly trained personnel are needed to carry out the observations, which differ quite drastically from those of the classical pollution biologist that have been in vogue for the past several years. In addition to the initial, omnipresent need for money, two things are necessary for research of this type: (1) favorable attitude of administrators, and (2) trained personnel to do the work.

A somewhat different approach to the assessment of the effect of pollutants on aquatic animals has been proposed (Brett, 1958), which embodies the concepts of resting and active metabolism and scope of activity as originally described by Fry (1947). In this method the resting metabolism (oxygen consumption) of "normal" or control animals (fish were suggested as assay animals) is measured and then their maximal rate of oxygen consumption is determined by subjecting the animals to some type of forced activity (e.g., swimming against a current). The difference between the two rates of oxygen consumption is designated as scope for activity or performance capacity. The experimental animals from the polluted environment are tested similarly and any change in performance capacity noted. This procedure is based on the assumption that no matter what environmental stress is placed on an animal, it will be reflected in a change (increase or decrease) in normal metabolic rates. In properly conducted experiments the effect of a pollutant could be assessed by stating its effect on either the active or resting metabolism. An alternative way of expressing the results would be to indicate the degree of change in the performance capacity of the assay animals as the result of exposure to a toxicant. With the first approach one would be faced initially with the need for setting up norms for the metabolic levels associated with a variety of actions and normal functions in the assay animals. With respect to a decrease in performance capacity one would have to arrive at some decision as to the magnitude of change that must occur before any toxic effect could be considered to be acting on the animals. Despite several difficulties in this approach to the study of pollution, the method does have advantages.

In general, less highly trained personnel are needed to carry out research and probably the overall expenditure of funds for equipment would be lower than that for the studies noted above. A respirometer similar to that designed originally by Fry (1947) could be used for fish studies and, since this apparatus would not be extremely cumbersome, it might be possible to conduct both laboratory experimentation and studies in the field. With the advent of dropping mercury and polarographic oxygen electrodes, which could supplement or supplant the use of the dependable Winkler procedure, measurement of the dissolved oxygen content of water should present no obstacle.

If it would be impracticable to conduct as elaborate an experimental program as that outlined above, one could resort to a method similar to that described by Fromm (1958) for the determination of the effect of toxic materials on the general metabolism of fish. Also, with some aquatic and/or air-breathing macroinvertebrates, measurement of their oxygen consumption could be carried out with the conventional Warburg apparatus or a constant pressure respirometer such as that designed by Reineke (1961).

Both the specific physiological and the general metabolic approaches to the study of pollution as discussed above have their advantages and disadvantages with respect to the type of personnel needed, amount and type of research equipment and facilities necessary to carry out this type of research, etc. From the standpoint of comparative physiology and medicine relatively little would be gained from the "general metabolic" approach; however, many benefits may accrue from physiological studies. For example, Knoll and Fromm (1960) reported that hexavalent chromium is not concentrated in muscle tissue of fish to any significant degree, hence human consumption of this portion of fish taken from water contaminated with 2.5 milligrams of chromium per liter or less would probably represent no serious health hazard. Dr. R. J. Collins (Collins, Fromm, and Collings, 1961) has presented data obtained in our laboratory on the excretion of chromium by the dog. This report provides some information as to the way both tri- and hexavalent chromium are excreted by a mammal and is apparently the only report dealing specifically with this topic. It might be added that the use of isotopic chromium clinically is becoming more and more widespread and any information concerning the mode of excretion of this material is desirable. In addition, Dr. J. R. Hoffert (Doctoral thesis, Mich. State Univ., 1962) has made a thorough study of the metabolism of hexavalent chromium by the nucleated red cells of the painted turtle. He has developed methods for tagging these cells and has provided procedures that can be utilized in the study of blood and blood-vascular changes of lower vertebrates in response to a stress (pollution) situation.

If it is assumed that pollution studies utilizing a physiological approach are desirable, we are immediately faced with the problem of where and by whom will such studies be carried out. The author is of the opinion that a great deal of encouragement should come from the Environmental Health Section of the U. S. Public Health Service. This agency or group has jurisdiction over large sums of federal monies and, if properly informed, they should be quite competent to stimulate and subsidize research work of this type in various agencies and organizations. Industry should also bear its share of the responsibility in pollution abatement. Among other things, industry could contribute to research in water pollution by setting up additional industrial laboratories, by subsidizing new and currently active programs in universities and various state and federal agencies, and by providing facilities to aid pre- and post-doctoral fellows in their research.

In some states the Department of Conservation has highly trained personnel who could carry out programs such as those suggested above. Those departments not having people in this category should give some serious thought to retraining individuals, making it possible for certain employees to return to school to work on graduate degrees. If this is not feasible these departments should consult with research workers at various universities or possibly hire them in an advisory capacity or as director of a summer research program. Many college researchers would probably welcome the opportunity to work at state fish hatcheries or act in a supervisory capacity, in some cases on a gratis basis.

Federal laboratories such as those operated by the U. S. Fish and Wildlife Service and the Robert A. Taft Sanitary Engineering Center, as well as state agencies other than those noted above, should also be involved in these research activities. Cooperation between various agencies is desirable. In Michigan, for example, we have biologists associated with the Institute for Fisheries Research (closely allied with Univ. of Mich.), the state Water Resources Commission, Institute of Water Research (Michigan State Univ.), and the Michigan Department of Conservation, all of whom cooperate in various research projects. As long as all persons concerned remain dedicated to the task of providing usable and adequate water supplies for our present and future generations, cooperation can be achieved even though some competition between the different agencies may exist.

It has not been the purpose of this paper to provide ready answers to the manifold problems encountered in water pollution. Rather, it is a plea to broaden our experimental design for the study of water pollution; more specifically, a plea for instituting additional research based on a concern for the physiologic responses of aquatic organisms to non-acutely lethal concentrations of pollutants. There is no suggestion that proven, fruitful areas of research be discontinued, but that still another aspect be added to the entire study. In the "medical assault" on cancer, for example, many points of attack are utilized - surgery, chemotherapy, radiation, hormonal treatment, virus studies — all play a very important role and few people have concerned themselves with worrying about which approach is best. In pollution studies, the "assault" should likewise be many-pronged — studies of stream ecology involving benthic macro-invertebrates and fish, measurement of diatom or periphyton populations and their growth, TL_m determinations, and, as suggested here, experiments utilizing physiological and histopathological techniques. No single approach will provide all the answers but, taken as a whole, biological research can and must provide data on which estimates and recommendations for water quality criteria can be based.

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ANALYSIS OF EXPERIMENTAL MULTIVARIABLE ENVIRONMENTS RELATED TO THE PROBLEM OF AQUATIC POLLUTION

D. F. Alderdice *

INTRODUCTION

The influence of quantitative environmental variables on a true response η may be considered from the relationship

$$\eta u = \phi(x_{1u}, x_{2u}, \dots x_{ku})$$

for the u^th^ combination of levels where the k associated variables $x_1, x_2, \dots x_k$ are varied over $u = 1 \dots N$ combinations of levels (Box and Wilson, 1951).

If the associated factors are considered as a finite set, the biological response of an animal to a pollutant tested at levels of the factors of the set may be written, for example,

$$Y_p = f\ \{x_1, x_2, x_3\}$$

where Y_p = measured response to a fixed concentration of a pollutant. $\{x_1, x_2, x_3\}$ = a set of three environmental variables, which will operate according to their presence or levels on Y_p. Possible variations in the operation of the finite set may be stated as

$$\{x_1, x_2, x_3\}, \{x_1, x_2\}, \{x_1, x_3\}, \{x_2, x_3\}, \{x_1\}, \{x_2\}, \{x_3\}, \{\xi\}$$

where the absence of variables in the set refers to a coded zero or "standard" level, the empty set $\{\xi\}$ being standardized with respect to all associated variables.

It follows that none of these response systems may be identical in measured effect, that is,

$$Y_p\{x_1, x_2, x_3\} \neq Y_p\{x_1, x_2\} \neq \dots \neq Y_p\ \{\xi\}$$

In other words the response to a fixed concentration of a pollutant may vary according to the combinations of levels of associated variables impressed on the response system.

Thus, for a set of three associated variables operating on the response, estimated by Y_p, the function ϕ may be approximated by

$$Y_p = b_0x_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3$$

a polynomial of order 2 embodying the mean effect, main effects, quadratic effects, and cross-products. Estimates of the b's, the regression coefficients, would be obtained experimentally from orthogonal designs where

$$b = \Sigma xy/\Sigma x^2$$

Extension of factorial schemes to the composite designs of Box and Wilson (loc. cit.) and the methods of Plackett (see Box and Wilson) for incorporating additional observations into initial designs sequentially, provide extremely useful versatility in exploring the response surfaces that may be defined by the polynomial.

Estimates of Y_p may be obtained by appropriate substitution of levels of the variables in the fitted polynomial. However, a better appreciation of the manner in which the associated variables modify Y_p may be obtained by examination of the response surfaces computed from the geometric equivalent of the polynomial

$$Y_p - Y_{ps} = \lambda_{11}X_1^2 + \lambda_{22}X_2^2 + \lambda_{33}X_3^2$$

where Y_{ps} = response at the center, or region of stationary response of the geometric configuration

λ_{ii} = eigenvalues defining the rate at which Y_p changes in moving away from the center in the direction of X_i

X_i = canonical form of the original variables, the transformation translocating and rotating the x-coordinates of the experimental design to correspond with the X-axes of the response configuration.

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RESULTS

Studies of this nature have been carried out experimentally with juvenile coho salmon (*Oncorhynchus kisutch*). An example of the use of these methods was made with reference to cultured pre-smolt coho tested from mid-January to mid-February 1962, averaging 7.35-centimeter fork length and 4.31 grams in weight, at a time approximately 2 months prior to normal seaward migration.

Tests were conducted on samples of 10 fish per trial at 3 milligrams per liter of sodium pentachlorophenate over levels of salinity (°/oo), temperature (°C), and dissolved oxygen (mg/1) in a 15-trial composite factorial design. Response to each of the trial conditions was measured as median resistance time in minutes. A further eight trials were performed for improved definition of the principal axes of the resulting response surfaces. Levels of salinity, temperature, and dissolved oxygen are coded in the following manner:

Factor	Levels			Base level	Unit
x_1 Salinity, °/ooS	1	5.5	10	5.5	4.5
x_2 Temperature, °C	2	4.5	7	4.5	2.5
x_3 Dissolved oxygen, mg/liter	3.5	5.5	7.5	5.5	2.0

where

$$x_i = \frac{\text{factor level - base level;}}{\text{unit}} \quad \text{e.g. } x_1 = \frac{S - 5.5}{4.5} = -1,0,1$$

The responses obtained over two replicates (Trials 1 through 15) and additional trials (16 through 23) are listed in Table 1.

Calculation of regression coefficients from the trials of Table 1 provides the following estimated, with their standard errors

$b_0 = 111.1478 \pm 7.97$ $\qquad$ $b_{22} = -14.5269 \pm 6.72$

$b_1 = 6.9025 \pm 4.42$ $\qquad$ $b_{33} = -7.3060 \pm 6.81$

$b_2 = -7.0395 \pm 4.51$ $\qquad$ $b_{12} = 0.4918 \pm 5.02$

$b_3 = 2.4953 \pm 4.49$ $\qquad$ $b_{13} = 0.1298 \pm 4.69$

$b_{11} = -3.8914 \pm 4.86$ $\qquad$ $b_{23} = -2.0013 \pm 5.17$

Therefore

$$Y_p = 111.1478 + 6.9025x_1 - 7.0395x_2 + 2.4953x_3 - 3.8914x_1^2 - 14.5269x_2^2 - 7.3060x_3^2 + 0.4918x_1x_2 + 0.1298x_1x_3 - 2.0013x_2x_3$$

Table 1. COMPOSITE FACTORIAL DESIGN FOR 3 FACTORS EACH AT 2 LEVELS, INCLUDING TRIALS AT THE CENTER AND ON THE AXES OF THE FACTOR SPACE AT ±1.215 UNITS FROM THE CENTER (TRIALS 1-15). TRIALS 16 THROUGH 23 WERE PERFORMED IN SUPPORT OF INITIAL FINDINGS.

Trial	Associate factor levels*			Average response time, minutes
	x_1	x_2	x_3	Y
1	-1	-1	-1	83
2	-1	-1	1	89
3	-1	1	-1	62
4	-1	1	1	71
5	1	-1	-1	92
6	1	-1	1	114
7	1	1	-1	93
8	1	1	1	99
9	0	0	0	100
10	1.215	0	0	107
11	-1.215	0	0	108
12	0	1.215	0	88
13	0	-1.215	0	98
14	0	0	1.215	98
15	0	0	-1.215	98
16	1.215	-1	1	111
17	1	-1.215	1	113
18	1	-1	1.215	100
19	1	0	1	100
20	2	-1	1	104
21	1	-1.400	1	68
22	1	0.600	0.380	72
23	0.340	0.200	0.125	138

* x_1 — Salinity, °/oo. x_2 — Temperature, °C. x_3 — Dissolved oxygen, mg/liter.

A point of stationary response is computed at the locus

$x_{1s} = 0.8890$ $\qquad$ $x_{2s} = -0.2421$ $\qquad$ $x_{3s} = 0.2117$

equivalent to

9.50°/oo S $\qquad$ 3.89°C $\qquad$ 5.92 mgO_2/liter

The response at this point is calculated as 115.3324 minutes. The polynomial can be reduced to canonical form

$$Y_p - 115.3324 = -3.8852X_1^2 - 14.6689X_2^2 - 7.1702X_3^2$$

The signs of the components on the right-hand side of the equation indicate that the response decreases in moving away from the point of stationary response in the direction of the X-axes. The stationary point is concluded to be a point-maximum or optimum.

The response surfaces, accordingly, are ellipsoidal and are illustrated in Figure 1 as constructed from the canonical equation. Five sections were taken through the ellipsoid in the plane of X_1 and X_2 at $X_3 = \pm 1.204, \pm 1$ and 0. With combinations of levels of salinity, temperature, and dissolved oxygen limited to those that provide 87 percent (100 minutes) or more of the optimum response (found at the center, S, of the ellipsoid where $X_1 = X_2 = X_3 = 0$), planes were constructed in Figure 2 with the 87 percent response loci as the axial boundaries of each plane or ellipse. Combinations of salinity, temperature, and dissolved oxygen levels selected on these planes ($X_3 = \pm 1.204$ are point limits in the direction of X_3 at $X_1 = X_2 = 0$), which provides at least 87 percent of maximum resistance time to 3 milligrams of sodium pentachlorophenate per liter, may be approximated from the calculated relationships in Figure 3.

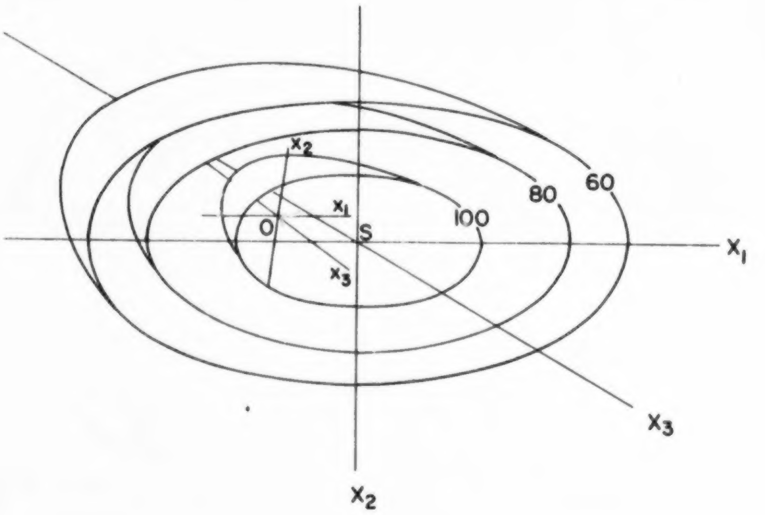


Figure 1. Response surfaces in terms of median resistance time in minutes for coho salmon juveniles exposed to a 3-mg/l concentration of sodium pentachlorophenate at various levels of salinity (X_1), temperature (X_2), and dissolved oxygen (X_3). The configuration is ellipsoidal and is shown sectioned through the X_1 and X_2 axes at $X_3 = 0$. The X-axes refer to the response configuration; the x-axes, with center 0, are those of the factor space investigated and $X = f(x)$. Three envelopes are illustrated around the locus of maximum resistance at S, enclosing loci equal to or greater than 100, 80, and 60 minutes median resistance time, respectively. The optimal value at S is calculated as 115.33 minutes.

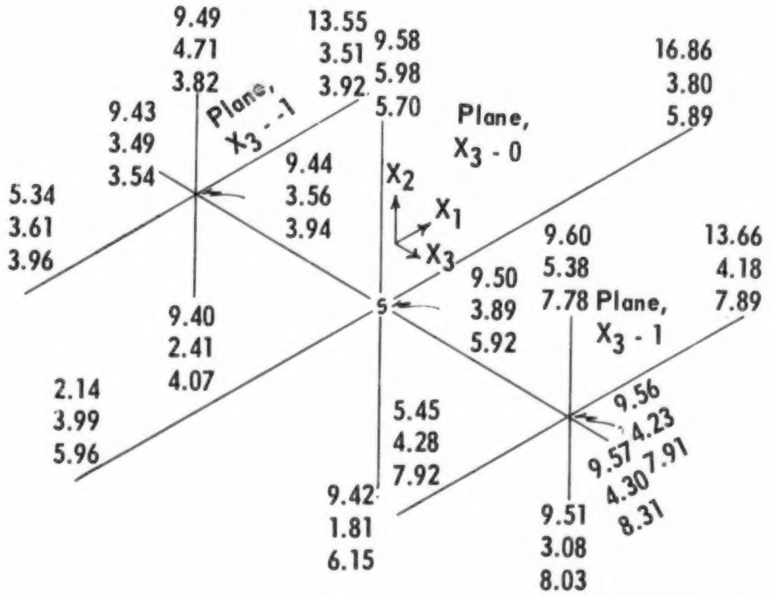
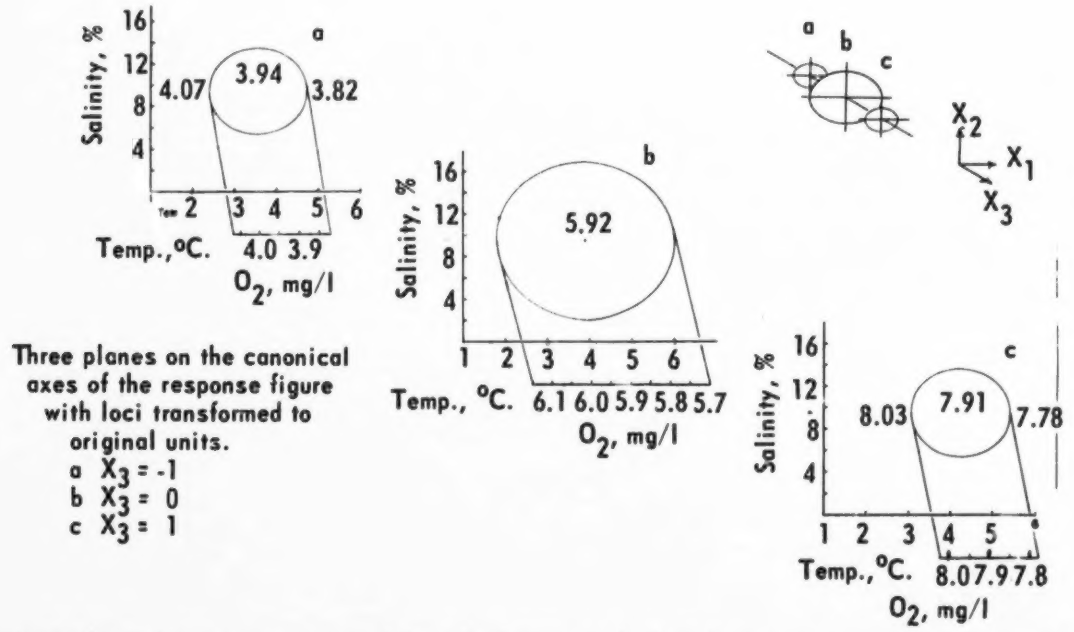


Figure 2. Boundary conditions for the 100-minute median resistance time envelope (Fig. 1) providing loci equivalent to 87 percent of the response at S. The figure is constructed by taking 3 planes through the X_3-axis of Fig. 1 at $X_3 = \pm 1$ and 0, calculating corresponding values of X_1 and X_2 at which the response is 100 minutes, and transforming the X values to original units. Each triplet of figures indicates: upper—S°/oo, middle—°C, lower O_2, mg/l.



Three planes on the canonical axes of the response figure with loci transformed to original units.
a $X_3 = -1$
b $X_3 = 0$
c $X_3 = 1$

Figure 3. Combinations of salinity, temperature, and dissolved oxygen which at 3 milligrams of sodium pentachlorophenate per liter are calculated to provide 87 percent or greater of the response at S on the planes at which $X_3 = \pm 1$ and 0. The axial limits at which $X_3 = \pm 1.204$, $X_1 = X_2 = 0$ are not shown (9.43°/ooS; 3.49°C; O_2, 3.54 mg/l; 9.57°/ooS; 4.30°C; O_2, 8.31 mg/l of Fig. 2).

DISCUSSION

Considerable research effort within the past several decades has been applied to describing functional capacities among fishes. Studies of metabolic rates, primarily through measurements of oxygen consumption, have more clearly shown the manner in which changes in the aquatic medium may influence the internal state of the animal and associated dependent activities. Homeostasis mechanisms have been recognized in poikilotherms as providing a measure of independence from the state of the external medium in allowing the continuation of vital activities. Although individual contributions to knowledge of functional capacities are numerous, the most recent critical evaluations and compilations of Fry (1947), Prosser (1955, 1958), Bullock (1955), Wanberg (1956), Brown (1957), and Prosser and Brown (1961) must be mentioned.

Concomitant with the measurement of capacities has been consideration of the underlying question regarding their ecological significance, not only with reference to survival of the individual, but also with respect to maintenance of the species. For example, while the lethal temperatures forming the boundaries of the zone of temperature tolerance (Fry, *loc. cit.*), may be defined with precision, they bear only a gross relationship to habitat and geographical distribution. The ecological significance of temperature preferenda is also unclear. Temperatures permitting optimum scope for activity, however, appear to have ecological significance (Brett, 1956).

In spite of the major accomplishments in the description of functional capacities among fishes, there remain large gaps in our knowledge. This is because the biologist, faced with the practical field problem, is still unable to predict with accuracy or certainty the results of many changes in the environment where none of these is obviously acute. It is not the intention here to subject existing estimates of functional capacities to critical scrutiny, although Winberg (*loc. cit.*) clearly indicates that such scrutiny is justified in some areas. Rather, it appears a bridge of understanding is needed between functional capacity and the evaluation of the significance of these capacities to meet environmental change.

A partial key to the more critical evaluation of capacities is suggested to lie in their examination within a framework of physical and chemical factors more closely approaching those of the normal and inescapably complex environment. The constrained experimental conditions under which capacities are usually evaluated present a highly simplified and hence a typical environment compared with the varying circumstances to which the animal may be subjected naturally. Moreover, homeostasis mechanisms may only partially compensate for variations in the external medium, resulting in more stressful or less stressful areas within the zone of tolerance. Furthermore, if several or more factors vary, with normally viable limits for each factor, an associated response may reflect variations in viability or capacity diverging from levels that would be expected by single-factor estimation. The fact that interactions in biological systems are the rule rather than the exception would support this argument. Evidence of such interaction is widespread in the literature (e.g. Downing and Merkens, 1957; Alabaster, Herbert and Hemens, 1957; Lloyd, 1961).

Related to the problem of investigating the complex environmental situation is the consideration of experimental design. Simultaneous examination of several or more variables quickly increases the complexity of investigative procedure. Efforts have been made to reduce the complexity of experimental procedures by instituting standard methods with reference to such variables as experimental water, age, size, species, temperature, and oxygen concentration. If one is strictly concerned with the pharmacological definition of comparative toxicity, such measures are well founded. If the end result of experimentation, however, is the assessment of probable effect of environmental change or adulteration on associated fishes, the requirements for definition will not be standard but will be concerned with the characteristics of the species and of the water it inhabits.

The system under examination becomes one not of single variables but of multiple variables in which functional capacities may be expressed in the environmental situation by the association or interaction of a number of factors, all in some manner defining the absolute level of effect. Functional capacities then delimit the ranges of variables of known concern, and their action as associates will determine the response to the total complex.

Hence, in the example presented, of primary interest is the fact that the response of the test fish to the toxicant varied according to the levels of the several variables simultaneously impressed on the test fish. Furthermore, the existence in this case of a particular combination of levels of the three associated factors that provides maximum or optimum resistance also provides a means of qualitative assessment of the various levels of associated factors that may influence the response to the toxicant. Around the response center, computed at 9.50°/ooS, 3.89°C, and 5.92 milligrams of oxygen per liter for pentachlorophenate, extend envelopes of equal levels of response provided by alternate combinations of levels of the three factors considered. Those enclosing the ellipsoid providing 87 percent of the optimum response or greater range from approximately 2 to 17°/ooS, 2 to 6°C, and 3.5 to 8.5 milligrams of oxygen per liter.

The response surfaces were constructed on the basis of acute biological assays. One reasonably might question whether or not the same relationships would hold at lower concentrations of the pentachlorophenate. Although corresponding response surfaces have not been evaluated at other lower levels of the toxicant, the response to combinations of salinity, temperature, and dissolved oxygen has been determined on linear pathways cutting through the response surfaces (Alderdice, unpubl.). In these cases the same pattern of response has been obtained at other pentachlorophenate concentrations.

It is not suggested that all potentially pollutional materials would yield response surfaces similar to those developed in the example. The manner in which the animal responds to associated environmental variables may very likely be determined by the influence of the particular pollutant on functional abilities associated with the response.

A further complication found in the study of juvenile coho salmon is that the response to the three associated variables tested is not constant with age. The locus of the optimum of the response configuration changes with age in the test fish (Alderdice, unpubl.).

Discussion of the significance of the results obtained requires consideration of the errors involved in their estimation. The methods of analysis employed were proposed by Box and Associates for problems in which experimental error is small. Adequacy of fit of the data to a polynomial is then judged by a comparison of the residual mean square, after fitting of the constants in the polynomial, with the experimental error variance. In the case presented here, the polynomial is a good representation of the data in the factor space investigated, although the experimental error variance is greater than is theoretically desirable. It follows that the response surfaces constructed and loci estimated on these surfaces should be considered as best estimates based on the existing data.

The interaction terms of the polynomial would be zero only if the axes of the factor space investigated and computed response surfaces were coplanar. Although the calculated interaction terms are smaller than their standard errors, there is no reason to suggest that these estimates are, in fact, zero. The calculated quantities are, therefore, retained as the best estimates available from the data. The terms indicate factor dependence, biologically not unexpected. Although the degree of dependence in the example (Figure 2) suggests the interaction effects could be ignored in related studies on other ages of juvenile coho, a high level of biological significance can be attached to the dependence between the factors studied.

When compared with 3^3 factorial designs, the composite design employed provides estimates up to second order in half the number of experimental trials, with some loss of sensitivity in provision of the estimates. In this study it was necessary to work in the shortest practicable experimental intervals, as the test animals were known to show appreciable changes in response with growth. With the availability of computer services, the extension of the designs and methods of analysis employed to more extensive factorial arrays should be possible, with little increase in computational difficulty.

A one-factor-at-a-time approach to the study of the effects of the three variables employed would have the best opportunity to succeed only where there is no factor dependence. Otherwise, an estimate of the locus of optimum response would probably be obtained, in this case on a salinity ridge below the center, after a considerable number of experimental trials. In this respect, the methods are an effective means not only of describing the response surface in the factor space investigated, but also for indicating the direction to proceed toward higher response levels.

Although there are errors involved in the estimation of the response surfaces, these errors are not unique to the methods employed. Since the environment may act as a complex, interpretation of the manner in which the response may be altered by the complex should conceivably be investigated by methods that consider the association and interaction of the various factors forming the complex. This is considered a fundamental argument for the use of the methods employed.

The response configuration, once derived, provides considerable insight into the manner in which many possible combinations of the associated variables may influence the response. One is led to question the validity of biological assay data developed under constrained experimental levels of associated environmental variables, when such data are used to interpret the significance of related pollutional changes in the aquatic environment. If physical and chemical characteristics of waters vary seasonally or geographically, such variations may have a marked effect on resistance patterns of associated fish populations exposed to even a constant pollutional load. The combination of levels of associated variables providing optimum resistance and the manner in which their variation affects the response to particular effluents or water-borne contaminants may help define conditions, locations, or times in which contamination is associated with high or low potential stress among resident fishes.

If the natural limits of variation in the levels of associated environmental variables are known or may be estimated for a particular field situation, those providing the lowest survival potential, as calculated from the response configuration, should be used as background levels when attempting any experimental determination of "safe levels" of concentration for a pollutant for which the response surfaces have been estimated. Although the estimation of "safe levels" is another problem entirely, its relationship with associated factor levels is unmistakable. The ability of the experimenter to consider the problem of toxicity simultaneously with associated physical and chemical properties of the water that may influence such estimates is regarded as being of first importance.

From the point of view of preventing or minimizing a potential pollutional problem, the relationship between associated environmental factors and their effect on the response to a pollutant may initially define the most likely locations in which such a problem would be minimized with respect to certain water quality alterations.

The existence in a response configuration of a point or area of maximum resistance to a particular pollutant in terms of normally tolerated levels of environmental variables provides for a definition of qualitative changes in water in terms of the resistance of selected resident species. Water quality as a function of biological response might then be monitored with reference to the contaminant defining the problem.

Remedial measures could be taken with regard to pollutional load when alteration in levels of associated variables indicates a deterioration in response to a locus on the response surfaces outside of an envelope such as that defining combinations of water characteristics providing 90 percent of the optimum response.

Where the level of an associated variable, such as dissolved oxygen concentration, varies outside of normally tolerated levels, the considerations dealt with still apply. The problem becomes a special case only in that the factor in question exerts more influence on the multivariable system than when it operates within tolerated levels, and under these circumstances may limit responses at all combinations of levels of the other factors in the complex.

To summarize these arguments consider, for example, the problem of deciding on the best location for a proposed industrial development with respect to its water-borne wastes. The suggested steps toward a decision are as follows: 1. Select one or more economically important species of fish and determine their response surfaces for the contaminant in question, with reference to environmental variables considered to be of importance. 2. Define the 90 percent response boundaries. 3. On the basis of these findings decide on the location best satisfying the biological requirements. 4. At this location obtain the known or estimated range in variation of associated physical and chemical characteristics of the water and choose the set providing the lowest response level within the 90 percent response boundary. 5. With the levels of this set as background levels, determine the concentration-response relationship for the contaminant in question and provide a limiting concentration with a statistically defined "cushion" (e.g. $x_0 - 4\lambda$ of Gaddum, 1956). 6. Monitor the actual situation chemically and biologically, as illustrated, against the probability of changes' in water quality exceeding the defined limits of variation.

With reference to such questions as "How much oxygen does a fish need?" or "What is the highest concentration of a particular pollutant that may be tolerated?", it appears that the answers are relative ones--relative to the other factors and their levels, which may be impressed simultaneously on the particular animal considered. It is suggested that the arguments presented may assist in a better comprehension of the significance of functional capacities in meeting environmental change, particularly when considered with reference to changes in water quality found in the polluted environment.

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THE BIOLOGY OF WATER TOXICANTS IN SUBLETHAL CONCENTRATIONS*

Charles G. Wilber †

INTRODUCTION

The problem of evaluating the significance of water pollutants is at times discouragingly complex. The uses of water are so varied as to demand a bewildering number of viewpoints in pollution assessments.

In the past, most studies have involved the killing effects of pollutants on selected living organisms. Useful information has been derived from such studies. The evaluation of a water toxicant in terms of kill or no-kill, however, is somewhat circumscribed in effectiveness. Many delicate responses of organisms are never observed. All too often crude data, based on 50 percent kill or on time to kill 50 percent of the test animals, are used to arrive at rather broad conclusions.

With the advent of a wide spectrum of pollutants that are poured into waters from many sources, it seems essential that a more sophisticated approach be made to the problem of toxicants in water. The need for studying sublethal concentrations of toxicants for the entire lifetime of the test organism has been clearly emphasized. [1] The disrupting effects of minute concentrations of certain toxicants in water on the normal embryological processes suggest that studies involving several generations of the test organism should be carried out. [2, 3]

In this paper I propose to discuss critically the relationship between a toxicant and its biological effects and to suggest a theoretical model for the assessment of water toxicants in sublethal concentrations.

ALL-OR-NONE RESPONSES

In the evaluation of the relationship between a toxicant and the physiological effect it produces, two factors or variables must be considered:

1. Concentration of the agent,
2. time of exposure.

The latter variable is suprisingly often neglected; all too frequently it is considered for only relatively brief periods. In addition, the physiological effect to be produced and recorded is usually death. The measure of the effect is ordinarily the *time* required to kill one half of the experimental animals or the *dose* to kill one half of the experimental animals' in a specified time.

In either event, one is faced with an all-or-none response. The biological significance of the animals' not dying is ignored; their value to the elucidation of mechanisms of action of toxicants is minimized. Similarly, one is left uninformed concerning the biological significance of the animals that died.

The following general equation is useful to express the relationship of dose to time: [4]

$$Ct = K + a\,t^{\,b+1} \qquad (1)$$

where C is the concentration of the toxicant in appropriate units; t, the time of exposure; K, a constant related to species susceptibility; a, a constant related to the magnitude of rate of detoxication; and b, a constant related to change in rate of detoxication. It is evident that if the constants K, a, and b are known, the relation between dosage (Ct) and time (t) to produce a specified physiological effect may be determined.

A graphical method of analysis makes estimation of the various constants relatively easy. Equation (1) is rearranged as follows:

$$Ct - K = a\,t^{\,b+1} \qquad (2)$$

If logarithms of both sides are taken, a straight line is invariably obtained when log (Ct - K) is plotted against log t; the slope of the resulting line will be (b + 1); log a is equal to the intercept on the log (Ct - K) axis when t = 1. If log-log paper is used to plot these values, all terms may be read directly from the chart without conversion to logarithms.

K is ascertained by plotting Ct against t on regular coordinate paper. If K is difficult to locate (it is the intercept on the Ct axis when t is zero), because of the curvature of the resulting line, a plot of log (Ct - K) against log t, which gives the straightest line, will approximate K adequately.

The importance of the time factor in evaluating possible toxicant hazard is worth repeated emphasis. Legal considerations make it important for water pollution experts to have a clear grasp of the real role of time in modifying toxicological responses. [5]

If equation (1) is used to evaluate the biological effects of a compound suggested as a possible oil spill eradicator, some interesting results follow. The data are taken from published literature. [6] The numerical results from Chadwick [6] were rearranged to permit the calculation of the toxicant in milligrams per liter. Time in minutes is given and Ct was calculated in terms of milligrams per minute per liter. When time was plotted against Ct, K was found to be 2,300. (Ct - K) was calculated and plotted against

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time in minutes on log-log paper. The constant a was found to be approximately 37, which would suggest that the eradicator is not detoxified to any great extent. Table 1 gives the selected calculated data in tabular form. The physiological effect tested was death of all fish.

Table 1. BIOLOGICAL EFFECTS OF OIL SPILL ERADICATOR ON STRIPED BASS[6]

Concentration of agent, mg/liter	Lethal time, minutes	Ct, mg/min/liter	Ct - K[a]
50	60	3,000	700
16	300	4,800	2,500
10	600	6,000	3,700

[a] K = 2,300; a = 37.

It is necessary to point out that formulas involving the Ct = K relationship quickly lead to absurdities if taken seriously over wide ranges of values.[7] This kind of formula asserts that an infinitely small concentration of a drug will produce a specified action in infinite time and that an adequately large concentration will produce an instantaneous effect. Over very small ranges of concentration or time, the product Ct may be constant. Extrapolation is of questionable validity. The older literature on the subject is reviewed by Clark.[7] The straight-line relationship resulting from the use of the Ct = K formula is useful, but this analysis must be used prudently to avoid misinformation and error.

THE LD_{50} CONCEPT

If one is studying the toxicity of some compound to a test animal, the LD_{50} value is often used. The LD_{50} is the amount of toxicant in the test animals that kills 50 percent of those tested in a specified time. In tests of this kind, the toxicant is usually injected into the animals.

The danger and irrationality of using the LD_{50} concept for animals respiring in an aquatic medium should be obvious; the pitfalls are illustrated in a recent paper[8] in which the attempt is made to express the toxicity of 1-naphthyl N-methylcarbonate to goldfish in terms of the LD_{50}.

You will recall that the LD_{50} denotes the actual amount of toxic material that gets into the test animal. In the paper referred to,[8] the term LD_{50} is used to apply to the concentration of toxicant in the surrounding water. Such usage is meaningless--but worse, the data may be taken seriously by others.

It is obvious that if a toxicant is added to the air a man is breathing, the concentration of the agent and the time during which the subject breathes the poisoned atmosphere are both essential if one is to conclude anything about the biological action of the agent.

Similarly, as was pointed out previously in this paper, if the toxicant is added to water in which test animals are living, one must consider time of exposure if the conclusions are to have meaning. The term Ct, discussed previously, must be used. Following from this it is clear that the term LCt_{50} may be used and properly so; it is the product of concentration and exposure time that will kill 50 percent of the test animals.

The data on the toxicity of sevin® were rearranged and recalculated to give approximate results that included time of exposure as well as concentration in milligrams per liter. The results are given in Table 2. If the data are plotted on logarithmic-probability paper to give the best straight line, the value for the LCt_{50} if found to be about 78,000 milligrams per minute per liter. This value gives a more nearly accurate expression of the situation (e.g., the concentration of the toxicant acting for a given time) than a futile attempt to arrive at an LD_{50} value without knowing how much toxic material actually enters the body of the organism. There have been other reports, e.g., toxicity of dieldrin to fish, in which the LD_{50} is expressed as parts per million in the medium (Reference 15, p. 87). The meaning of such data is obscure.

Table 2. TOXICITY OF SEVIN ® TO GOLDFISH[8]

Ct, mg/min/liter	Percent dead
100,800	100
86,400	85
72,000	20
43,000	20

THE RECTANGULAR HYPERBOLA

For sublethal concentrations of toxicants in water, the dose-effect relationships that have been discussed here are of doubtful value. The dose-response curve encountered must frequently, when one is dealing with sublethal concentrations of agents, is the rectangular hyperbola. Direct experimental data support the theory favoring the hyperbola as a widely applicable generalization describing the effects of pharmacologically active agents on living protoplasm.[7]

As an example, the time-concentration curve for inducing systolic arrest of the isolated heart in the frog, with the toxic agent strophanthin, is described by the following equation

$$(C - 0.5)(t - 2) = K = 30 \qquad (3)$$

where C is concentration of drug in parts per 10 million and t is time in minutes. Likewise, the entrance of cresyl blue into *Nitella* is described accurately by a hyperbolic time-concentration curve.

The general function to be anticipated in testing sublethal concentrations of water pollutants will be of the type:

$$(X - a)(Y - b) = C \qquad (4)$$

where a, b, and c are unknown constants. Fitting a curve to points that fall along a hyperbola requires a modicum of ingenuity. There are two general methods for such curve fitting: the one suggested by Brown [10] and an alternate method advised by Hey and Hey. [11]

SALMON ORIENTATION

A good example of the hyperbolic nature of the dose-effect relationship at sublethal levels is found in the work of Jones and co-workers. [12] The data are particularly important because they involve meaningful biological responses to sublethal concentrations of water pollutants. Table 3 summarizes the rearranged and recalculated data. The curve that fits the experimental points surprisingly well is described as follows

$$(X - 120)(y - 20) = 1200 \qquad (5)$$

where X is concentration and y is the reciprocal of the mean percent avoidance (freedom of movement) of the salmon. Figure 1 illustrates the relationship.

Table 3. EFFECT OF SULFITE LIQUOR ON FREEDOM OF MOVEMENT OF CHINOOK JUVENILE SALMON

X, concentration, ppm	Y, mean percent avoidance	1/y x 1000, freedom of movement
2000	82.4	12.1
1000	65.4	15.3
500	44.5	22.5
250	25.1	39.8
125	11.4	87.7

A relatively simple method of fitting a hyperbola to experimental points has been suggested. [13] The data in Table 3 were so treated to fit the equation

$$y = k/x \qquad (6)$$

Table 4 shows the results obtained with the fitted curve

$$y = 10{,}000/x \qquad (7)$$

This curve may give a slightly better fit than does equation (5). The differences are not, however, great.

Table 4. CALCULATED VALUES OF Y BASED ON DATA FROM TABLE 3 AND THE EQUATION Y = 10,000/X

Let X equal	Then calculated y is
1,000	10
500	20
100	100
200	50
400	25
2,000	5
800	12.5
300	33.3

What is clear is the hyperbolic nature of the relationship between concentration of agent and biological response.

OYSTER TENTACLES

Another good example of the hyperbolic relationship obtaining between the concentration of a toxicant and its biological effect is found in the response of oysters to pulp mill pollution. [14] The delicate tentacles along the edge of the mantle in the oyster are sensory in function. These tentacles retract when various chemical agents come in contact with them. The latent period for the retraction is an indication of the irritating action of the agent in question.

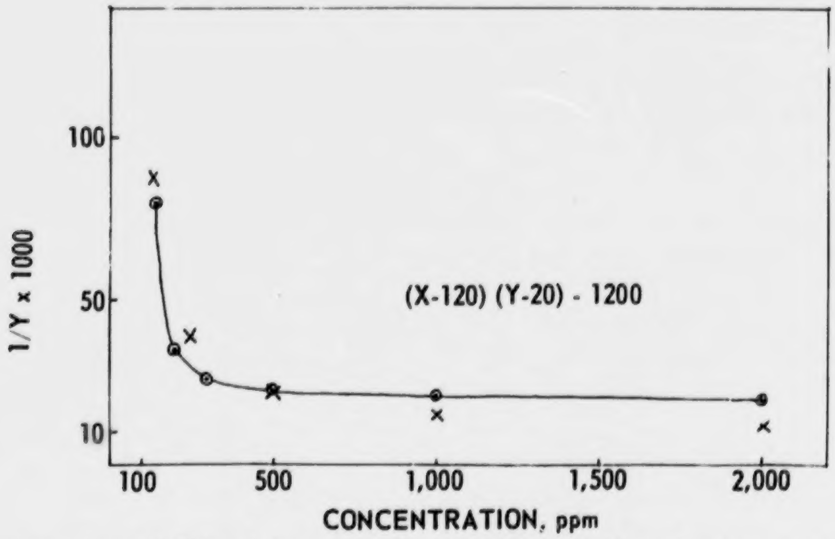

Figure 1. Avoidance response of Chinook juvenile salmon challenged with various concentrations of sulfite liquor. Data from Reference 12. X, experimental points; ⊙—⊙, fitted curve.

Published data [13] were taken and a curve in the form of equation (6) fitted to the experimental points relating concentration of pulp mill sulfite liquor to latent period for oyster tentacle retraction. A good fit is obtained as illustrated in Figure 2. Note that each experimental point is the average of 10 observations.

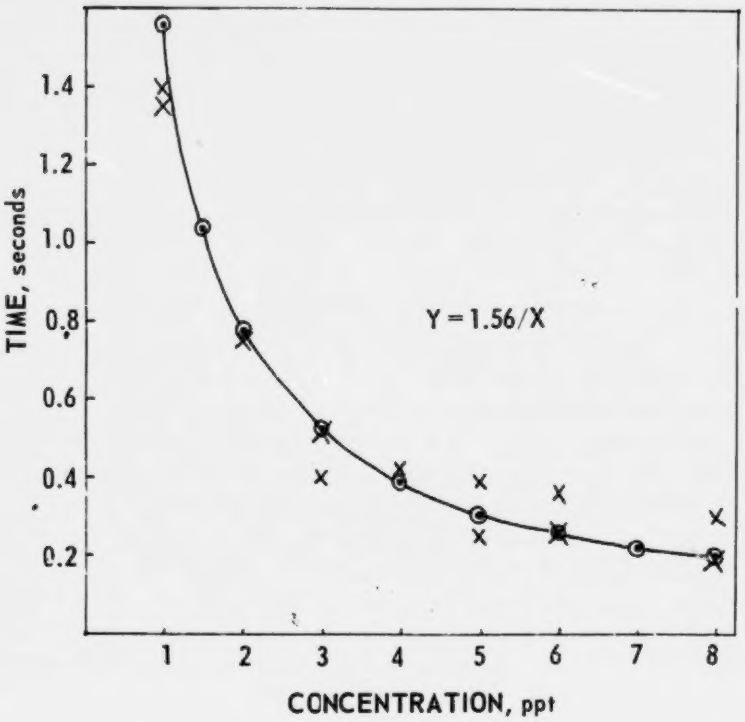

Figure 2. Relationship of concentration of pulp mill sulfite liquor in parts per thousand to latent period for retraction of oyster mantle tentacles in seconds.[14] The ⊙—⊙ hyperbola fitted by using equation y - k/x [13]; X, experimentally determined points, each based on 10 observations.

FISH BLOOD PRESSURE

In our laboratory we have attempted to use blood pressure changes in fish as an indication of biological action of toxicants in sublethal concentrations. The following experiment illustrates again the hyperbolic nature of the relationship between amount of agent and response. Dogfish, *Squalus acanthias*, were suitably prepared for recording blood pressure. Then various doses of lysergic acid diethylamide were administered by vein. The agent brought about a rise in blood pressure, which can be expressed by the following equation:

$$y = 95.6 - (5.5/x + 0.04) \qquad (8)$$

where y is the percent increase in systolic blood pressure over control, and x is the amount of the agent administered in micromols per kg of body weight. Figure 3 shows the plotted curve.

IMPLICATIONS

The examples presented show that the biological action of a toxicant in sublethal concentrations will vary with concentration in a hyperbolic fashion. As concentration of an agent in water increases, the time required for biological effect decreases to a measurable minimum. Above a certain concentration, C, time no longer decreases. This minimum time for biological effect is determined by the following (among other factors):

1. Passage of material through physiologically inactive shell of test animal,
2. peculiarity of toxicant itself,
3. circulation time from point of entry to site of action,
4. time for action at site,
5. build-up of effective internal concentration.

The last factor involves the rate of detoxication.

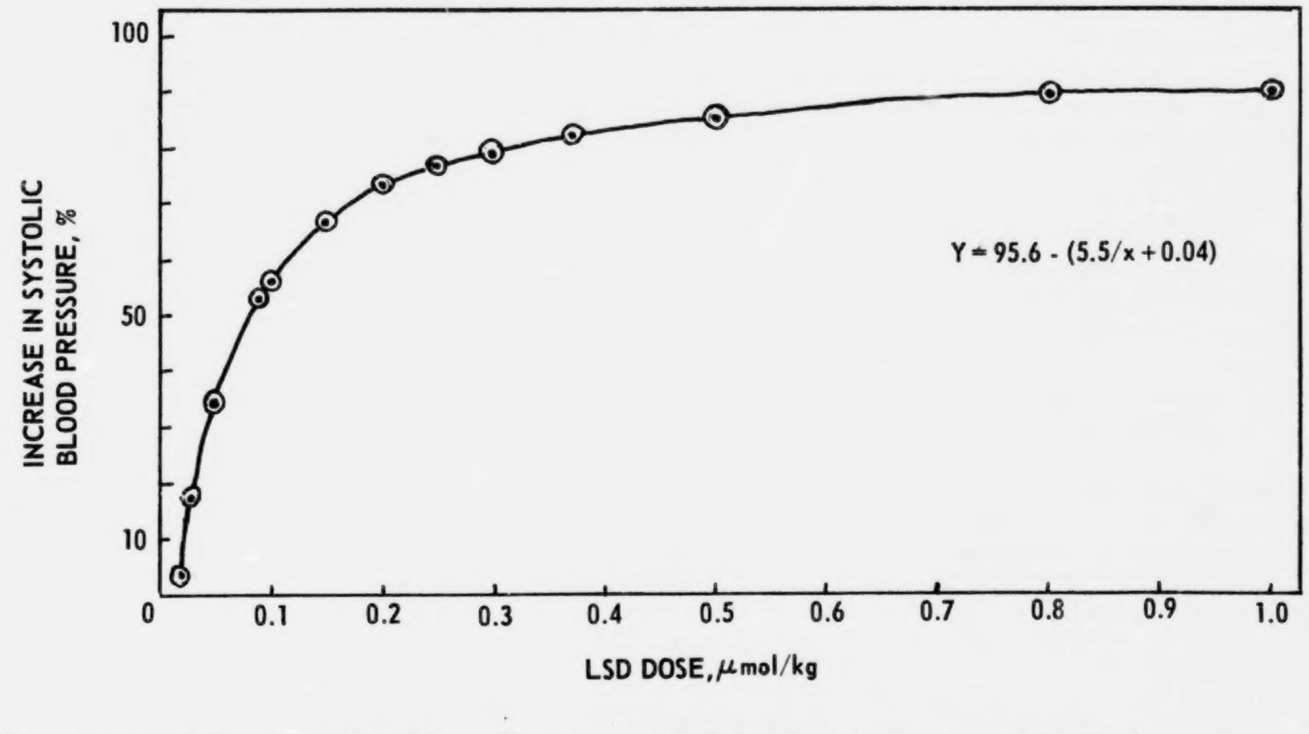

Figure 3. Hyperbolic relationship of dose of lysergic acid diethylamide to increase in systolic blood pressure as percent of control level in the dogfish, *Squalus acanthias*.

As concentration decreases, a point is reached at which no effect follows even if the toxicant acts for infinite time. At this point, C_o, the test animals exposed to the toxicant live as long as the controls, show no modifications of behavior from that of controls, and are virtually in the same biological state as the controls. Theoretically, then, C_o is biological zero concentration insofar as the responses of the test animal are concerned for all toxicants other than carcinogenic chemicals. C_o is determined by rate of detoxication and rate of loss of agent, through hydrolysis, adsorption, or other ways.

To emphasize the biological action of a toxicant, one can plot the reciprocal of time for effect against concentration. The resulting curve is a "curve of effectiveness" of the agent with respect to a given biological measure. This was done in Figure 1, which shows clearly the effectiveness of sulfite liquor in circumscribing the freedom of movement of young salmon.

BIOLOGICAL TIME

When long-term studies are made in which toxicants are used in sublethal concentrations it is important to recognize that biological time is a logarithmic phenomenon.[16] This fact has been called to mind by others.[9] It may partly explain why dose-response curves in which time is an element are of a logarithmic nature.

The logarithmic character of biological time must be kept in mind when one interprets long-term experiments with water toxicants. It is evident that the biological value and significance of a given time interval will not be the same at the beginning of a chronic exposure as it will be near the termination. Such a consideration might well be important in modifying one's conclusions.

CARCINOGENIC CHEMICALS

When one is dealing with agents that have the capacity to induce neoplasms in living organisms, a new dimension is added to toxicological studies. It is my contention that safe limits or maximum allowable concentrations have no meaning in reference to carcinogens.

Among the classes of chemicals that are known to be carcinogenic and are found as water contaminants are polycyclic hydrocarbons, azo dyes, amino compounds, halogenated hydrocarbons, and urethane. Some of these are capable of carcinogenic action in almost any tissue; others are tissue specific.

There is every reason to hold that carcinogenic agents combine with or exert profound action on various key cellular constituents "so that the heritable cellular physiology is altered in the direction of uncontrolled proliferation."[17*] It is clear, therefore, that one molecule of a carcinogen is all that is needed to combine with and act on a gene, plasmagene, or gene-enzyme complex to produce a cytological alteration that is heritable. That one alteration will then increase and multiply until it results in destruction of the host. In brief, this is the basis for denying, on theoretical grounds, any quantity but zero as the maximum allowable concentration of a carcinogenic toxicant in water that may be intended for human use, possibly for bathing, but certainly for ingestion. From a pragmatic point of view it may be impossible to demand that all water for human use be absolutely free of carcinogens. In that event, the toxicologist must be honest and admit the risk, which can then be accepted or rejected in the light of other economic, political, or emotional considerations.

When the effects of carcinogens on aquatic organisms are studied the all-or-none type of experiment is essentially meaningless. The test animals must be submitted to a thorough histopathological study. Great care must be exercised to insure that borderline responses are observed. The weights of key organs as related to body weight must be recorded. The constancy of organ weight-body weight ratios in normal fish is well known.[18, 19] The fact that water contaminants can bring about changes in these ratios has been recorded,[20] but this useful method of studying water toxicants in sublethal concentrations has not been used to best advantage.

In such assessments especial attention must be paid to liver and, if possible, kidney weights; for liver and kidney weights are those most frequently affected by a toxicant.[21]

GENERAL SUMMARY

The few points made in this paper may be summarized briefly.

1. In an evaluation of the lethal effect of a water toxicant *time* is a critical variable. The product concentration x time, Ct, is important as a measure of the lethal action of an agent.
2. Toxicants in sublethal concentrations are biologically important. The relation of the concentration of a toxicant, under these circumstances, to the biological effect is best described by a rectangular hyperbola and not by a straight line.
3. For carcinogenic agents there is theoretically no acceptable maximum concentration other than zero.
4. The use of organ-body weight ratios is a promising method for evaluating the effects of water toxicants in sublethal concentrations. It should have wider use.

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*Reference 17, p. 868.

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EFFECTS OF SUBLETHAL CONCENTRATIONS OF ZINC AND COPPER ON MIGRATION OF ATLANTIC SALMON

John B. Sprague *

In 1960 there was an unusual disturbance of the spawning migration of adult salmon in the Northwest Miramichi River, New Brunswick, Canada. Of the salmon moving upstream during the open-water season, 22 percent returned downstream through a counting fence maintained by the Fisheries Research Board of Canada. Previously there had been little downstream movement from June through October, the usual operating period of the fence. For example, in the previous 6 years the number of salmon falling back averaged only 1.5 percent of those moving upstream, and this figure never exceeded 3 percent in any one year.

The apparent cause of this disturbed migration was sublethal pollution of the river by zinc and copper. This resulted from increased activity during 1960 at a base-metal mine on a tributary river. Waters of this tributary were at times lethal to fish, and bioassays showed that zinc and copper alone were responsible for the toxicity. The bioassays were interpreted on the basis of toxicity determined in the laboratory and analyzed by the "Toxicity Index" method, which will be described further on.

Figure 1 shows that downstream movements of salmon during 1960 occurred when zinc concentrations were highest in the river. Zinc is used as a measure of the total pollution by copper and zinc. Salmon movements are summarized by 5-day periods for simplicity. As usual, there was a "spring run" and a "fall run" of fish, with heaviest migration during or after freshets. The concentration of zinc also increased during freshets. Interpolations of the amount of zinc have been aided by daily measurements of water level and by samples taken on different days in the polluted tributary.

During 1961, pollution from the mine was somewhat reduced, and the total downstream movement of salmon was 14 percent of those going upstream. To pinpoint the "safe" level of pollution for salmon migration, water samples for chemical analyses were taken daily at the counting fence.

To express the zinc and copper pollution as a single number, a method was used that was recently developed at the Water Pollution Research Laboratory in England (Dept. Sci. Indust. Res., 1961). Their work on the toxicity of metals to rainbow trout (loc. cit.; Lloyd, 1960) was spotchecked in young Alantic salmon, with excellent agreement. The variation in threshold or incipient lethal concentrations with water hardness has been described (Dept. Sci. Indust. Res., 1961).

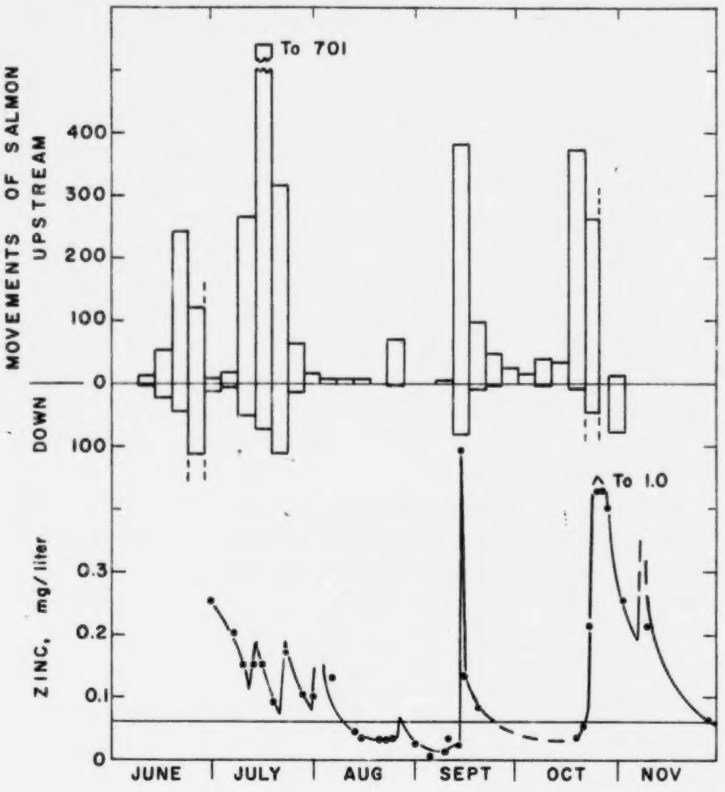

Figure 1. Degree of metal pollution compared with disturbed salmon migration in the Northwest Miramichi River in 1960. The abnormal downstream movement coincides with increased pollution as measured by concentration of zinc. The apparent correlation of heavy upstream movement with increased pollution merely indicates that both tend to occur during freshets. The numbers of salmon were recorded at the counting fence located at Curventon, New Brunswick, and were summarized by 5-day periods. Extensions to the columns by broken lines indicate that more fish may have passed during times of high water when the fence was partly inoperative.

To make use of this method, the incipient lethal concentrations of zinc and of copper were calculated daily for the Northwest Miramichi River on the basis of water hardness. The actual concentration of zinc in the Miramichi was then expressed as a fraction of the incipient lethal concentration. In this paper the resulting number is called the "Toxicity Index" for zinc, and a value greater than 1.0 indicates a lethal concentration. The Toxicity Index for copper was calculated in the same way. These two values were added together to obtain a single Toxicity Index for zinc and copper.

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In the Northwest Miramichi River, metals were usually in sublethal concentrations, and the Toxicity Index was accordingly less than unity. Changes in the Toxicity Index during 1961 are correlated with salmon movements in Figure 2. Inspection of this figure indicates that a Toxicity Index of approximately 0.15 is the maximum "safe" level for migration of salmon. Expressed as a percentage, this would be 15 percent of the lethal concentration. A consistent correlation with salmon movements was not obtained if any of the three factors, zinc, copper, or water hardness, was ignored in estimating the degree of pollution.

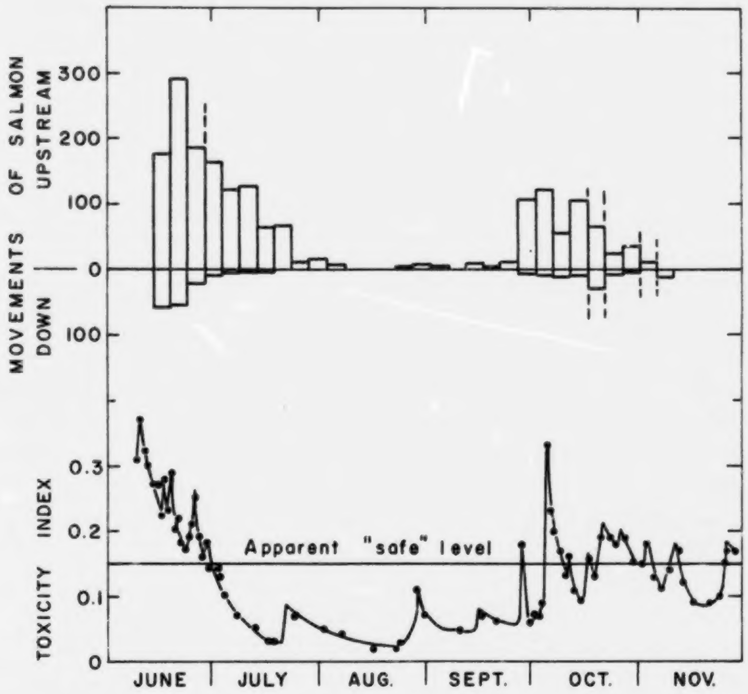

Figure 2. Degree of metal pollution compared with disturbed salmon migration in the Northwest Miramichi River in 1961. The effects of zinc and copper have been integrated and expressed by the Toxicity Index, as described in the text. A Toxicity Index of 1.0 indicates lethal action of young salmon after long exposure. A value of about 0.15 seems to be the maximum "safe" level for undisturbed salmon migration. Explanation of salmon movements is given in Fig. 1.

This work is being repeated in the Northwest Miramichi River in 1962 to test the estimated "safe" Toxicity Index of 0.15. At the time of writing there are not enough data from 1962 to confirm or negate this estimate. However, preliminary laboratory results indicate that young Atlantic salmon avoid concentrations of zinc with a lower Toxicity Index.

As an example of the amounts of metals involved in a Toxicity Index of 0.15, some figures can be given for water having a total hardness of 20 mg/l as CaCo$_3$. The incipient lethal concentrations would be 0.7 mg/l of zinc, or 0.05 mg/l of copper. Multiplying these by the Toxicity Index of 0.15, the estimated "safe' levels would be about 0.11 mg/l of zinc alone or about 0.008 mg/l of copper alone. If both 0.11 mg/l of zinc and 0.008 mg/l of copper were present, the Toxicity Index would be twice the "safe" level, that is, 0.15 + 0.15 = 0.30.

I would like to thank Dr. C. J. Kerswill for making available the data on movements of salmon past the counting fence. Dr. Kerswill is in charge of salmon investigations for the St. Andrews Biological Station of the Fisheries Research Board of Canada. Members of his staff helped in many ways, including the collecting of water samples.

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EFFECTS OF COOLING WATER FROM STEAM-ELECTRIC POWER PLANTS ON STREAM BIOTA

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"All the rivers run into the sea; yet the sea is not full; unto the place from whence the rivers come, thither they return again." Ecclesiastes 1:7.

Our problem is: What do we do to a river between its source and the sea? Among many other things, we dump heated water into rivers. The subject of this section of the third seminar is the release of heated waters into rivers, lakes, and estuaries. This subject should not be separated, however, from the other subjects under discussion. Without an ecological viewpoint all our findings and our painstaking work will go very much astray. Water cannot be separated into artificially heated and natural-temperature water, and water temperatures cannot be separated from all the various types of water pollution and the biota of the region.

This report at best can only suggest methods of approach to a big problem, a very big problem. The electrical power industry requires great amounts of water as a coolant. This industry is increasing at the annual rate of 10 percent in the United States, 12 percent in Britain, and, I am told, 50 percent in Russia. A careful estimate of thermoelectricity production in the 1980's in the U.S.A. is 2,000 bkwh (billions of killowatt hours). This will require about 200 billion gallons of water per day of which about 6 percent will be used for boiler makeup water and 94 percent for cooling water.

The annual runoff of water for the United States amounts to about 1,200 billion gallons per day; thus in about a quarter of a century about one-sixth of all runoff waters will be needed for cooling and makeup in steam-electric plants. This use added to all other uses--household, industrial, dilution and digestion of wastes, recreation, etc.--certainly points up a problem, perhaps one of the biggest ever attacked by American scientists and engineers. There is some chance that technological advances may reduce the amount of cooling water needed by the electric power industry per unit of production, but not very materially. After all, man does not repeal natural laws that are not laws in the human sense but only conditions we find in nature, in this case the cooling ability of water in reference to its specific heat.

At this point the term "thermal pollution" should be discussed. The term does not fit. "Pollution" is derived from the Latin "polluere," which means "to make dirty." Do heated water effluents make a river or a lake dirty? They may, but usually they do not. If the ecology of the water is known and thoroughly understood the word pollution attains a different meaning. It may mean increased basic production, lessened production, or no production and a sterile environment.

Among the 50 states of the U.S.A. there is little conformity in laws designed to control heated water. In some states, heat is called a polluting substance; it surely would take an Einstein to prove this in court. Many states have no laws regulating the release of heated water. In others the release of heated water is not governed by laws but by rules and regulations issued by a sanitary water board or some similar state agency. Since rivers generally flow between states and across state boundaries, confusion often reigns. Without a federal law regulating the release of heated waters, states with straddling boundary rivers and lakes must cooperate for control measures.

Intelligent control of the problem must be based on surveys of effects of release of heated water at all plants and complete surveys of possible effects of heated waters at all new plant sites. A working plan for such surveys is gradually evolving from work already done. Why should state or federal agencies lay down hard and fast rules without knowing the effects or the expected effects in a particular area? Surveys of this type would be initially expensive, but cheap in comparison to degradation of the water supply and constant litigation that might ensue.

Most steam-electric plants in America use direct flow of cooling water from a river, lake or estuary into a plant and back into the body of water without any attempt to cool or recirculate. In 1959 and January of 1960, through a grant from NSF†, I observed methods used to meet this problem in England and Wales. Through necessity, they have controlled the output of heated water better than we have. About 40 percent of their steam-electric stations use estuarine water, which creates problems of corrosion of condenser tubes by salt water and in some cases excessive growth of vegetation in areas receiving the heated water effluent. Since no part of the British Isles is over 50 miles from a coastline, estuarine water and sea water are used for cooling much more than in America. The remaining 60 percent of the steam-electric stations use river water; one-half of these use direct flow and the other half use the so called "beehive" natural-draft cooling towers.

Although these natural-draft cooling towers have been suggested for use in America, they probably would not work well here. England's climate is much more equable than ours. The cooling towers would be least efficient during periods of very high temperatures and humidity during an American summer when greatest cooling would be needed. And during low temperatures of winter the towers may fill with ice (this did happen in one tower in England). The cooling of water by evaporation is a consumptive use. Along 50 miles of the River Trent, steam-electric stations and cooling towers are so abundant that 20 percent of the mean flow of the river is evaporated. Pollutants are thus concentrated in the river, causing a problem.

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† National Science Foundation.

Other problems result from the use of cooling towers, either natural draft or forced draft. At times they produce a heavy fog in the area of the station. Of course a "wee bit of extra fog" would not be noticed in Britain. Sometimes glaze ice forms on roads and all other surfaces for several miles around an evaporating tower; this rarely happens in England, but much more often in northern U.S.A. Cooling towers may be necessary in certain areas to hold temperatures within reasonable limits; however, their limitations and objectionable effects should be realized.

In England, regulation of heated-water discharge and selection of sites for new plants are done by a planning board of the Central Electricity Generating Board in conjunction with the River Board concerned. This is another striking difference between British and American procedures. Our rivers and watersheds are much larger than theirs. Moreover, our rivers commonly form state boundaries or flow across state lines and are subject to varying regulations. England's River Board system subjects a whole watershed to uniform regulations after long, detailed, and continuous study.

Although most of our laws and regulations concerning water are founded upon English Common Law, British barristers have sometimes cited court cases from America to uphold a water use in England.

For 5 years, the Lehigh Institute of Research studied ecological effects of heated-water discharge into the Delaware River at Martins Creek. This research was supported by the Pennsylvania Power and Light Co. Because the Delaware River in the area studied is a surprisingly clean river with almost no pollution, it was an excellent experimental river. As is usual in research, many preconceived ideas were found to be wrong. The effects of heated-water discharge upon the river biota were not always as expected. The findings and research methods employed in this study may be of interest and value in planning and executing similar projects at other steam-electric stations. They can be divided roughly into the following fields: Plankton, periphyton, macroscopic invertebrates, rooted aquatic plants, filamentous algae, fish population studies, temperature studies, and chemistry of the river water. Brief remarks follow concerning each of these fields.

Plankton: Of the various methods of collecting river plankton, slow filtration through a millipore filter was found to be the best method. Because river plankton drifts with the current, however, and may be in and out of the study area in a matter of a few minutes, it was decided that little information could be gained from plankton studies in a river of this rapid-flowing type and these studies were discontinued.

Periphyton: Periphyton includes those organisms that cling to surfaces, usually stones on the bottom. Most plankton organisms are also included in the periphyton. A modification of the Caterwood Diatometer was used to assay the influence of heated water on periphyton populations. This instrument is essentially a plastic boat designed to float just below the surface and holds regular microscope slides behind a plastic water shield. Certain organisms, mostly algae, cling to and develop on the slides. Other organisms including protozoa, diatoms, etc., invade this habitat, and within a week, there is a representation of the periphyton biota. These slides may then be examined fresh, or stained and kept as a permanent record.

The study of the periphyton was very interesting and worthwhile. High temperatures tend to restrict the number of species present in an area, and may encourage high basic production.

Macroscopic Invertebrates: Invertebrates such as insect larvae, flatworms, round worms, segmented worms, mollusca, and crustaceans make up part of the food chain for large, catchable fish. With certain limitations, the Surber square-foot sampler seemed to be the best type of collection apparatus. This area has fast-flowing water over a rough, rubble bottom with some sand banks and mud banks. The usual Eckmann Dredge sampling techniques were very ineffective.

The macroinvertebrate fauna correlated well with the periphyton. The numbers of species in the heated-water zone were greatly reduced during summer months. The standing crop of these organisms in some cases was increased during winter months. Also, under conditions of highest temperatures the standing crop remained constant or was reduced. Midges, Tendipedidae, seemed to be the most tolerant invertebrates in the heated-water area. At the section of the river where complete mixture of heated water and normal river water occurred, fresh-water clams, *Unio*, were often very abundant. Other aquatic invertebrates tend to build up populations during the winter months and undergo reduction during the summer months. The area is constantly reseeded by drifting eggs, larvae, and adults from the river flow.

Rooted Aquatics: Rooted aquatic plants were exterminated in the river by the great Dianne Flood of August 18, 1955. Their recolonization was watched from 1956 until 1960. At first it was thought that these plants could not survive in the heated-water area; however, during the summer of 1960 a pond weed, *Potomongeton*, was found growing well in the heated-water area with water temperatures ranging from 95° to 100°F. We assumed that this was due to selection of heat-resistant types. This observation should lead to further research on tolerant types of organisms in areas subjected to heated-water discharge.

Filamentous Algae: In a river like the Delaware, great amounts of filamentous algae develop in back bays and shallow regions close to shore during late summer and early autumn. An increase in river stage of a few inches usually occurs in late September or early October. This carries great amounts of algae down the river. Old river men speak of this as "the grass going out." Green filamentous algae tend to grow more rapidly in areas of heated water during spring and early summer, but also tend to die off before autumn. In place of green algae, blue-green algae develop in great amounts during the warm months.

Certain species of blue-green algae, primarily *Oscillatoria*, we found were the most heat-tolerant organisms. They were observed growing well at temperatures up to 115°F. In heated-water areas during the summer, blue-green algae may make up most of the biomass. For a number of reasons overgrowth of blue-greens may under certain conditions be objectionable. They do not seem to be eaten by other organisms as much as green algae and rooted aquatics, they tend to overgrow themselves, with consequent release from the bottom and formation of large clots or masses of dead and dying blue-greens that float downriver. Blue-greens are good nitrogen fixers and when in normal abundance seem to aid in the basic productivity; however, when excessively abundant, they tend to die off and raise the biochemical oxygen demand of the river. Blue-greens clog filters in municipal water supplies and release foul odors and fishy tastes when they decay. Some are actually poisonous.

For some unknown reason blue-greens do not appear troublesome in similar situations in England. This may be because English river waters do not reach temperatures as high as ours, even at heated-water outfalls. Excessive heat in America may inhibit competing green algae and allow overgrowth of blue-greens, but this does not happen in England.

Fish Populations: Fish were taken by seining, trap nets, fyke nets, trot lines, electro-fishing, and angling. Scuba-diving also produced valuable assessment of fish populations. In a small lagoon connected with the discharge canal, rotenone was used with excellent results. This part of the study indicated that most of the fish species present in the area are attracted to and invade the heated-water area from late September until early June. During the hot summer months fish leave the heated-water zone. Forty species of fish were caught in the heated water during the study. Attraction to heated water has been observed in England and has been reported frequently in America. This adds to the recreational value of these areas, since angling can be continued throughout the winter when there is little or no fishing in other areas. We found little evidence that heated waters produce more fish or better growth rates; they simply attract fish and the fish actively feed throughout the colder months of the year. Almost without exception the fish taken in the heated-water zone were healthy and well fed. In general they were larger than those of the same species taken in the normal river. This may be due to the active feeding throughout the winter months, or to a greater attraction of fully developed fish for warm water.

We built an experimental fish flume for the study. Twenty-four species of fish were tested in the flume for the Median Lethal Tolerance Limit of heat. Results indicated that if fish were acclimated over a period of time to high temperatures the lethal temperature would be considerably above (3° to 4°) that expected as a result of previous experiments. There was a definite critical temperature for each species above which that species could not survive. The most resistant fish species was the killifish, *Fundulus diaphanus*, which survived 103°F.

Fish are good index organisms in polluted areas and in heated-water areas. Our work suggests that if time or economic reasons necessitate a short-time incomplete survey, fish populations should be studied first. If fish of the usual species present in a river or lake invade the suspected area and remain there, pollution and other disadvantageous conditions cannot be very severe. On the other hand, if local species are reduced in numbers or kind, a thorough investigation should be made.

Temperature Studies: Many temperature traverses of the river below the station were made over a period of 4 years; most of these were from a railroad bridge below the plant, some by boat below the railroad bridge. A thermistor held in a metallic capsule at the end of a flexible cable was a very useful and accurate instrument for this purpose. Three temperature-depth profiles of the river were also made with the "rope trick." A 600 - foot length of rope was stretched across the river and pulled as tight as possible with a tackle block. This served as a guide line so that temperatures could be taken every 6 feet across the river and at every 2-foot level from surface to bottom.

These measurements gave a fair picture of the dispersion and mixing of heated water with the normal river water. Almost complete mixing occurred at a point about 1 mile below the confluence of the discharge canal and the river. Before the construction of diversion jetties to aid mixing of heated and normal water, the heated water tended to cling to the shore on the Pennsylvania side for a much greater distance.

We often observed from the railroad bridge a very clear demarcation between the heated water and the normal river flow, usually marked by a line of foam. The excellent papers presented by C.H. Stranderg indicate that the path of flow of heated water in a river can be recorded by photography from the air and by infrared sensors. This would seem to be a very significant advance in the study of thermal discharge, which should be correlated thoroughly with the physiography of the study area, the chemistry of the water, and its ecology.

Chemical Studies: Water chemistry was studied throughout the course of field observations. Only dissolved-oxygen concentrations are mentioned here, since the other values recorded seem to have little to do with the heated water. We anticipated a serious loss of dissolved oxygen due to heating of the river water. On most test dates this was not true. An average dissolved-oxygen loss of 0.5 ppm was observed. Similar observations have been made in England and in other parts of America. This unexpectedly low loss may be due to the great turbulence of cooling water as it leaves the discharge structure. In the affected zone of the river, dissolved-oxygen values ranged from 4.3 ppm to 10.9 ppm.

Suggestions for further work:

1. Plant sites should be studied both before and after installation of generating systems. Release of heated water is becoming a serious problem, a problem that cannot be met without adequate data, especially data from particular plant sites.

2. Discharge structures should be planned to provide as great water turbulence as possible.

3. Rivers and lakes may be deliberately modified by thermal discharges to provide additional recreational fishing during cool-weather periods.

4. Cooling towers may be necessary to certain areas, but their advantages should be weighed against their disadvantages.

5. Research on the effects of thermal discharges into rivers, lakes, and estuaries should be continued indefinitely.

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THE CONTRIBUTION OF BOTTOM MUDS TO THE DEPLETION OF OXYGEN IN RIVERS, AND SUGGESTED STANDARDS FOR SUSPENDED SOLIDS

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The title of this brief introductory review is a portmanteau holding a number of implicit questions. A clear statement of these questions will be of assistance in enabling us to assess their importance and to judge how far we have gone towards answering them. These questions are:

1. Do bottom muds contribute to oxygen demand in rivers?
2. To what extent is this oxygen demand exerted by products of dissimilation in the sediment layer?
3. To what extent are bottom muds resuspended in the flowing water?
4. Do they then exert an oxygen demand?
5. Can we limit bottom deposits by imposing suspended solids standards on effluents?
6. What rational criteria can we apply to such standards?

Even if the author's commission had called for it, a definitive review of the topic is impossible at the present time. Perhaps rightly, this matter seems to have been very largely ignored, and what literature there is does not, unfortunately, lead to a consistent conclusion. The author has, therefore, set himself the task of drawing together the results of some of the more important investigations as they bear on the six questions.

On the face of it fairly firm answers of "yes" can be given to questions (1) and (4), but even these questions are well worth discussion. The other questions are still open.

The bottom deposits that are built up in a receiving water vary in location, composition, and quantity with:

a. The character and amount of waste matter entering the water (including, of course, material eroded from the catchment);

b. the hydraulics of the body of water;

c. the rate and nature of decomposiiton of organic matter in the deposit.

If the supernatant water contains dissolved oxygen, aerobic conditions will exist only at the surface layer of the sediment because the diffusion of oxygen is normally so slow that the lower layers remain anaerobic. Decomposition of the lower layers will, therefore, be anaerobic, while the upper layers will be stabilized aerobically. The term "benthal decomposition" has been applied to this combination of anaerobiosis and aerobic stabilization.

DO BOTTOM MUDS CONTRIBUTE TO OXYGEN DEMAND IN RIVERS?

The Royal Commission on Sewage Disposal exhaustively examined the whole question of sewage disposal and river pollution in England and Wales between 1898 and 1915, and published many voluminous reports. In particular the lengthy Appendix to the Eighth Report (1913) bears on this matter. On several occasions (e.g., pp. 116, 118) the authors of this Appendix state categorically that fouling of the stream bottom is responsible for further deoxygenation of the water flowing over it. This comment is based on extensive observation of many English rivers by outstanding investigators. We can, I think, therefore, accept "yes" as the answer to our first question.

First, however, we must take notice of a complicating factor: suspended solids interfere with the dissolved-oxygen determination (Ruchhoft and Moore, 1940; Forth River Purification Board, 1959). The Forth survey showed that high concentrations of suspended solids reduced the apparent oxygen concentration by 10 to 25 percent. Nevertheless, even allowing for this, it is clear that river muds, if they do become resuspended in the stream flow, can make a substantial contribution to oxygen demand - but this is a topic to which we must return in looking at questions (3) and (4).

In accepting the affirmative answer to our first question it is worth noting that we are concerned with at least three cases that differ in the importance accorded to the likelihood of resuspension:

a. A nontidal river in which resuspension is possible only at times of increased river flow, i.e., freshet or flood;

b. a tidal estuary with such a very large water volume (and such a shape) that resuspension is unlikely to be appreciable in relation to the total water body except in the case of violent storm;

c. a tidal river in which resuspension is likely at each flood tide, e.g., the upper portion of the Forth estuary (Forth River Purification Board, 1959; Collett, 1961).

It will be readily appreciated that the relative contributions to oxygen demand of the settled deposit and of the resuspended solids will vary quite widely. It is likely also that the total contribution of the solids will be greatest where resuspension is greatest.

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TO WHAT EXTENT IS THIS OXYGEN DEMAND EXERTED BY PRODUCTS OF DISSIMILATION IN THE SEDIMENT LAYER?

Only a few workers have investigated this facet of the problem (Rudolfs, 1938; Baity, 1938; Fair *et al.*, 1941). Rudolfs studied the progressive benthal oxygen demand from sludge placed in carboys, the supernatant being drawn off periodically and replaced by tapwater. Fair and his colleagues at Harvard used a modification of Baity's apparatus to simulate the stream bottom conditions. Samples of sludge or river mud were placed at the bottom of stoppered glass carboys, and a continuous, controlled flow of water was passed through the carboys; it was directed horizontally across the surface of the deposit by a specially designed inlet and then flowed spirally to the water surface. The reduction in dissolved oxygen was measured daily, and the rate of flow, which was also measured, was adjusted to give a measurable difference between the dissolved-oxygen concentration in the inflowing and outflowing water. The average detention time in the carboys was 1 to 2 days. The aerobic and anaerobic phases of benthal decomposition were isolated, for purposes of comparison, by stabilizing separate sediment samples under essentially aerobic or anaerobic conditions.

Baity (1938) had found that the oxygen demand of sludge deposits was independent of the dissolved-oxygen concentration in the supernatant water, and this was borne out by the observations of Fair *et al* (1941). The most important conclusion of the Harvard investigators is that it is not the rate of diffusion of oxygen from the supernatant water into the organic sediment that controls the rate of benthal oxygen demand, but rather the rate of upward transport to the surface of the sediment of the oxidizable substances produced by anaerobic action within the deposits. Fair *et al.* (1941) found that the maximum depth for this upward diffusion was about 10 centimeters.

Parenthetically it may be worth noting that some observations made on the Mersey estuary (Department of Scientific and Industrial Research, 1938) bear on this matter. The main deposits of organic mud in the estuary were in the Stanlow Bank, which had been stable over many years. Samples of mud were taken at depths of up to 16-1/2 feet below the surface of the estuary bottom and were analyzed for organic matter. It was possible, from a study of the quinquennial survey charts of the Mersey Docks and Harbour Board, to give approximate dates to these various samples back to the year 1861. The analyses showed "that the amount of organic matter in the samples of material deposited in earlier years is approximately the same as in recent deposits containing the same amount of sand. There is thus no evidence that the mud mixed with these samples is significantly different in composition from the mud at present found on the surface at Stanlow Bank."

It seems clear, therefore, that the benthal oxygen demand is very much slower than the aerobic oxygen demand of the same material suspended in the flowing water. For example, the Royal Commission investigators showed that the oxygen demand of some samples continued with comparatively little diminution in rate for 2 years (Royal Commission on Sewage Disposal, 1913). Fair *et al.* (1941) applied their mathematical formulation to these last-mentioned data and showed that four sediment samples would take 15 to 25 years to become 99 percent stabilized.

In the Thomas estuary, which is deep and thus has a small perimeter per unit volume, the oxygen uptake at the surface of the mud represented only a comparatively small fraction of the total oxygen consumed in the estuary - about 8 tons per day or about 1 percent, out of an estimated total of over 1,000 tons per day. This oxygen uptake was measured in a form of respirometer in the Water Pollution Research Laboratory and in a larger in situ version of the respirometer. (Southgage, 1962, Ministry of Housing etc., 1961).

In addition to the work of Baity already mentioned, the Water Pollution Research Laboratory (D.S.I.R.) has made some laboratory and field determinations of the uptake of oxygen by mud deposits in the Thames estuary (see Table 1). For the laboratory determinations, an apparatus very similar to that of Baity was used; for the field determination, a similar technique was used by measuring the change of oxygen concentration in a stream of water passed over 0.25 square foot of mud enclosed in a special apparatus, which could be lowered to the river bottom Water Pollution Research 1953. Results are shown in Table 1. The same Laboratory, in the course of its studies of the oxygen balance in freshwater streams, has made an estimate of the "mud respiration" in unpolluted reaches of the River Ivel (Water Pollution Research 1961); this result is also shown in Table 1.

For many years the Freshwater Biological Assocation has investigated this matter in connection with its more extensive research on Lake Windermere. Undisturbed cores of the lake mud, obtained by means of the Jenkin sampler, were tested in the laboratory and gave typical oxygen-uptake rates of 0.2 of a gram of oxygen per square meter a day. At the same time, observation of the oxygen concentration in the hypolimnion, during the period in which the lake water was thermally stratified, showed the total oxygen demand of the hypolimnion to be 0.4 of a gram of oxygen per square meter a day. If the laboratory figure of 0.2 of a gram a day for the mud is accepted, it has to be assumed that there are considerable transfers of dissolved oxygen from one part of the hypolimnion to another. No such assumptions need be made if the mud uptake is, in fact, of the order of 0.05 of a gram of oxygen per square meter a day (Mackereth, 1962). More recently an investigation of Blelham Tarn has been put in hand. In the course of this investigation, large plastic cylinders have been sunk in the lake to isolate manageable volumes of the lake water; some of these cylinders are open to the bottom mud and others are closed at the base. The observations of oxygen concentration, which have not so far been analyzed, appear to bear out Mackereth's belief that the oxygen demand of undisturbed bottom muds is a good deal less than is shown by laboratory estimations. (Mackereth, 1962.)

Table 1. BENTHAL OXYGEN UPTAKE

Nature of tests	Oxygen uptake rate, g O_2/m^2 day	Reference
Laboratory determinations		
Sewage sludge 0.5 cm deep	1.84	Baity (1938); Fair *et al.* (1941)
1.0	2.89	
1.5	3.45	
2.0	3.77	
4.0	5.17	
Undisturbed cores from Lake Windermere	0.2	Mackereth (1962)
Thames mud 1 day 7°C	0.49	Water Pollution Research 1953
15	0.63	
25	1.22	
5 days 7	0.20	
15	0.39	
25	1.02	
Field observations		
Windermere mud	0.05^a	Mackereth (1962)
Thames mud		
30 min 16.5°C	0.88	Water Pollution Research 1953
19	0.98	
20	0.88	
River Ivel (unpolluted reach)	$2\text{-}5^b$ 2.0-5.0	Water Pollution Research 1961

[a] Estimated from the total hypolimnion rate of uptake of 0.4 g O_2/m^2 day - the theoretical value to give a coherent oxygen deficit curve in the hypolimnion without assuming unlikely transfers of oxygen from one part of the hypolimnion to another.

[b] "In most cases it has been assumed that the mud respiration represents the difference between that of the total community and that of plants." Most respiration data were collected at night.

TO WHAT EXTENT ARE BOTTOM MUDS RESUSPENDED IN THE FLOWING WATER?

The Mersey Survey (Department of Scientific and Industrial Research, 1938) observed that, in the upper estuary of that river, mud banks that had reached a height of about 20 feet above Liverpool Datum were relatively stable (hydraulically); we have already seen that this mud has collected for almost a century without becoming stabilized. Mud deposits at a lower level, however, are to a great extent resuspended on each tide (Water Pollution Research 1948).

At the other extreme is the upper part of the Forth estuary, from Stirling down to Bo'ness, where the soft, almost black mud is resuspended to a greater or lesser degree by each tide, aided by wind, where the water shoals over banks. At high tide and at low tide, slack water permits the solids from all levels to settle completely in the deep-water channel. (Forth River Purification Board, 1959).

Tarzwell and Gaufin (1953) have shown that, on the Great Miami River, the first high water after an extended period of low water picks up sludge beds and carries them farther downstream. As a result the zone where the dissolved-oxygen concentration is critically low is extended.

In nontidal rivers, it goes without saying that there is a downstream transfer of suspended matter. In tidal estuaries this cannot be taken for granted. It has been found in the Type estuary, for example, that the net movement of bed load is upstream (Cassie *et al.*, 1960, 1962). It is finally removed by dredging.

We shall show, in the next section, that the contribution to oxygen depletion made by organic solids in suspension is very much greater than that of the stationary bottom deposits. The degree of resuspension that can take place in any particular circumstances may, therefore, be of critical importance to the oxygen concentration in a river. This point is particularly emphasized in the Thomas estuary report (Ministry of Housing and Local Government, 1961).

Resuspension of bottom deposits is not, however, the whole story: bottom deposits are, for the time that they remain on the bottom, in effect oxygen-demand removed; any factors, therefore, that affect sedimentation in streams may well be relevant. It has been estimated that an appreciable fraction, possibly about a fifth, of the oxidizable matter (both soluble and insoluble) entering the Thames estuary is removed by deposition and dredging (Ministry of Housing and Local Government, 1961). Three matters investigated by the Water Pollution Research Laboratory (D.S.I.R.) may be of interest in this matter.

In the late 1940's the Laboratory investigated, for the Mersey Docks and Harbour Board, the effect of the discharge to the Mersey estuary of an alkaline sludge. This sludge, referred to as distiller blow-off (or DBO) sludge, arose from the manufacture of sodium carbonate by the Solvay ammonia process. It contains, in suspension, calcium carbonate, calcium sulphate, and calcium hydroxide with impurities from limestone and coke, and, in solution, high concentrations of calcium and sodium chloride, and small concentrations of dissolved calcium hydroxide and calcium sulphate. Laboratory tests showed that when sufficient sludge was added to a suspension of estuarine mud in non-saline estuary water to increase the suspended solids to 50 ppm, the settling rate was greater than that of a mud suspension alone with the same suspended-solids concentration. Where, however, the sludge was treated by carbonation before discharge, the settling rate was reduced. Further, the Mersey mud with carbonated sludge was less easily eroded than the mud alone or than the mud with untreated sludge. (Water Pollution Research 1948.)

In connection with the investigation of the River Ivel (Water Pollution Research 1961) already mentioned, it was shown that a sewage effluent prevented the deposition on snail shells or glass plates of calcium carbonate from the river water, which had a total hardness of about 290 ppm (as $CaCO_3$). The same sequestering effect was observed in the laboratory with carboxymethyl-cellulose. (Water Pollution Research 1959).

"It has been reliably reported that the concentration of suspended matter in the water of the Thames Estuary has visibly increased during the last 10 years, and it is suggested that the increased use of synthetic detergents during this time may be responsible." (Water Pollution Research 1960). To test this hypothesis in the laboratory, various concentrations of detergent-free river mud were suspended in a mixture of equal volumes of detergent-free sea water and tapwater. Settling tests were run in pairs, with one of each pair carrying a concentration of 1 ppm of a straight-chain alkylbenzene-sulphonate. "These results show that the effect of 1 ppm surface-active matter is to decrease the rate of settling of the mud for any given mud concentration. In one experiment in which the detergent concentration was increased tenfold, the effect on solids in suspension was roughly doubled." (Water Pollution Research 1960.)

The Mersey Survey showed that there was some interaction between sewage solids and suspended solids but that the net effect depended on the degree of mechanical flocculation and stirring (Department of Scientific and Industrial Research, 1938).

A rather higher velocity of flow is required to scour bottom deposits than is necessary to keep the material in suspension once it has been scoured. It goes without saying that the scour velocity depends on the nature, size, and specific gravity of the particles to be lifted, and on the Darcy-Weisbach friction factor. These criteria are used in the design of grit channels for sewage treatment plants, and Velz (1949) has analyzed grit channel experience in an attempt to define scour velocities in streams. He suggests that, to scour digested organic matter with a 1-millimeter particle diameter, a velocity of 1.3 feet per second would be necessary; he regards this as an extreme case. Fresh organic sludge can be resuspended at velocities of 0.6 to 1.0 foot per second.

Velz had an unusual opportunity to test his theory in studies on the Kalamazoo River made for the National Council for Stream Improvement. Two reaches with bottom deposit were separated by an intervening clean stretch. An examination of the river hydraulics showed that the channel velocity in the clean stretch would not drop to 0.6 foot per second until the flow dropped to 130 cubic feet per second. Hydrological analysis indicated that a runoff as low as this had a probability of only 5 percent. Both theory and observation demonstrated, therefore, that sludge deposit occurred only quite rarely. In the upper reach where deposits occurred, a channel velocity of 0.6 foot per second was attained when flow dropped to 700 cubic feet per second, a frequent occurrence. The reach was examined at a time when the runoff was 835 cubic feet per second and showed the presence of organic deposits without large accumulations. In the lower sediment stretch, large accumulations of stable organic material were found. For this stretch, channel velocities of over 0.6 foot per second were reached only when the flow exceeded 1,500 cubic feet per second, and flows of less than this for long periods gave rise to the extensive accumulations noticed. The lower reach was scoured only during freshets or floods.

It is dangerous to extrapolate from a single series of tests, but we may conclude from Velz' work that it is likely that channel velocities of 0.6 to 1.3 feet per second will be sufficient to resuspend organic bottom deposits in streams.

DO RESUSPENDED BOTTOM MUDS EXERT AN OXYGEN DEMAND?

There can be no doubt that the answer to this question is an unequivocal affirmative. This is borne out by such qualitative observations as that of Spencer (1962) who, in the 1930's, noticed that disturbance of river flow during constructional alterations to a bridge stirred up bottom deposits and caused a very marked reduction in the dissolved-oxygen concentration, resulting in fish mortalities. From the time of the Royal Commission onwards careful laboratory measurements have been made to assess the contribution of suspended organic solids to oxygen depletion; these we shall here examine.

"Observations have shown that solids . . . while in suspension, account . . . for a considerable proportion of the total oxygen uptake" in the Thames (Ministry of Housing and Local Government 1961). It has not yet proved possible to trace, with any degree of exactitude, the movement of sludge deposits in the

Thames, but it has been observed that "the change in position of the deposits with varying fresh-water flow affects considerably the concentration of suspended solids in estuary water in their neighbourhood, and this in turn influences the rate at which oxygen is taken up in different parts of the estuary." (Water Pollution Research 1952.)

First, however, it may be of value to record some analyses of bottom deposits so that we may assess their possible oxygen demand; such analyses are shown in Table 2. Although there was a very wide variation among the very large number of Mersey mud samples, the general carbon:nitrogen ratio was about 10.

The comparatively few data given in Table 3 are so diverse that it is difficult, if not dangerous, to draw any but general conclusions. Two conclusions, however, clearly suggest themselves. The first is that the potential oxygen demand of bottom deposits is very considerable if these deposits become resuspended. A closer study of the Connecticut River data suggests that, as may be expected, this demand (even when measured in terms of the initial volatile solids) is reduced as the deposits become "stabilized." Stabilization, in this sense, probably includes the results of two effects: Hydraulic scour, which would tend to reduce organic matter more than inorganic, and dissimilation, which would assist this differential organic breakdown.

Table 2. MUD ANALYSES (based on dry weight)

Material	Moisture, %	Volatile solids, %	Organic carbon, %	Kjeldahl nitrogen, %	O:N ratio	Sulphide (as S), %	Reference
Thames mud	51.0		1.92	0.214	9.0	0.160	23
South San Francisco Bay:							8
Reference			1.13			0.030	
Dumbarton Bridge-Calaveras Pt.			1.20			0.037	
Calaveras Pt. - Alviso Slough			1.43			0.041	
Coyote Creek			1.54			0.094	
Connecticut River deposits	45 to 97	2.50 to 14.4					12,16
Domestic sewage sludge	91.5	83		3.75			1
Sludge deposit	88.8	13.9		0.62			6
River mud (New England)	82.6	24.2		1.02			6
English river muds							
Anker	76.3	15.3		4.4 [a]			13
Avon	75 to 83	11.7 to 17.1		4.1-5.1 [a]			
S. Delph	46.7	11.0		1.3 [a]			
Canal	60.3	11.2		2.4 [a]			
Intertidal deposits, upper Mersey estuary	10.8 to 71.6	1.2 to 21.4	0.00 to 4.34	0.01 0.53	0.6 to 46.5	0 to 0.18	5

[a] Total nitrogen (averages) as % of volatile matter in dried mud.

The oxygen demands of sludge and mud solids when stabilized aerobically are collected in Table 3. For comparison it is worth noting that the initial benthal oxygen demands given in Table 1 for the sewage sludge (Baity, 1938; Fair *et al.*, 1941) range from 1.83 to 5.17 grams of oxygen per kilogram of volatile solids per day.

Although it is, perhaps, not strictly relevant to an answer to our question, it is interesting to note that the Royal Commission on Sewage Disposal (1913) made careful estimates of the 5-day BOD (at 18°C) of a number of settled sewages and sewage effluents, both with their suspended solids and after paper

Table 3. OXYGEN DEMAND OF SUSPENDED SOLIDS

Material	Dry solids, %	Volatile solids, % (DS)	Oxygen demand at 20° C		Reference
			Time, days	Demand, g O_2 per kg (VS)	
Connecticut River deposits [a] 1	22.3	3.53	4	46.7	12, 16
			6	57.8	
			10	81.7	
2	40.9	2.50	4	56.4	
			6	75.2	
			10	123.5	
3	11.3	14.4	4	84.2	
			6	115.8	
			10	179.7	
4	2.6	11.7	4	93.9	
			6	131.8	
			10	198.2	
5	7.3	6.1	4	62.0	
			6	68.0	
			10	90.8	
6	41.3	6.73	4	24.1	
			6	31.5	
			10	44.2	
7	41.6	6.08	4	28.1	
			6	35.2	
			10	49.0	
8	54.7	2.97	4	34.7	
			6	42.8	
			10	57.3	
9	22.0	9.68	4	80.9	
			6	116.8	
			10	155.5	
10	33.7	4.33	4	30.0	
			6	39.4	
			10	53.2	
English River muds [b] - Anker (304)	23.7	15.3	5	32.5	13
			650	205.0 [c]	
Avon (306)	24.6	11.7	6	16.1	
			650	144.0 [c]	
Avon (308)	16.6	17.1	5	86.7	
			650	324.0 [c]	
Avon (309)	22.2	12.8	6	110.2	
			650	248.0 [c]	
Chelmer (123 k)	53.3	11.0	5	36.4	
			650	174.0 [c]	
Canal at Berkhamsted (128 k)	39.7	11.2	650	128.0 [c]	
Sewage sludge	8.5	83.0	5	173.0	1,6
Sludge deposit	11.25	13.85	5	331.0	6
New England river mud	17.4	24.2	5	47.7	6
Thames mud from dredger hopper					
Barking reach	43.1		5	6.2 [d]	26
Gravesend reach	33.5		5	8.7 [d]	
Tidal basin, Tilbury	25.5		5	9.5 [d]	
Royal Docks	18.0		5	15.6 [d]	
Tilbury Docks	24.0		5	8.1 [d]	

[a] Oxygen demands determined in a Warburg respirometer.
[b] BOD measured at 18°C.
[c] Oxygen uptake measured in Adeney's respirometer (in still water except for sample 123 K).
[d] In g O_2/kg dry solids.

filtration; it was found that paper filtration reduced the BOD about one-half. (Parenthetically it is worth remembering here that, for this reason, the Royal Commission's BOD standard includes the BOD of any suspended matter.)

Table 3 does not include results from the Survey of the upper estuary of the Forth because these are not given in a form that can be immediately translated into the form of this Table. Samples of mud, exposed at low tide, were collected from four points in the estuary. Sufficient amounts of each mud were mixed with 6 liters of clean sea water to give a mixture containing 1 percent dry matter; each mixture was used to fill two sets of six 12-ounce bottles. The suspension in the first bottle of each series was immediately precipitated with alum and ammonia, and the supernatant was drawn off for the measurement of dissolved oxygen. The other five pairs of bottles in each series were incubated at 20°C; one set of each series was allowed to settle and the other set of each series was shaken twice daily. Each day a pair (1 shaken, 1 quiescent) of each series was withdrawn, treated with chemicals, and the supernatant drawn off for dissolved-oxygen determination. The results are given in Table 4. (Collett, 1961).

Table 4. OXYGEN DEMAND OF RIVER FORTH MUDS (COLLETT, 1961)

Location	Seawater only	Ladysneuk		Haugh		Cambus Harbour		Kincardine	
Dry matter, %		52.3		53.9		41.5		42.3	
Organic matter, % (DS)		10.8		10.5		18.0		17.2	
Oxygen concentration, ppm		Sh[a]	Qu[b]	Sh	Qu	Sh	Qu	Sh	Qu
Initial	8.02	7.72	7.72	6.06	6.28	4.53	4.64	4.45	4.08
1st day	7.98	5.52	5.88	2.68	3.88	0.47	2.86	Nil	2.08
2nd day	7.94	3.97	4.86	1.46	3.48	0.06	2.28	Nil	1.38
3rd day	7.74	2.97	3.65	1.05	3.26	Nil	1.64	Nil	1.47
4th day	7.67	1.59	3.45	0.10	2.63	Nil	1.26	Nil	0.85
5th day	7.61	1.03	2.20	0.07	1.02	Nil	0.54	Nil	1.16

[a] Shaken.
[b] Quiescent.

It will readily be seen from these results that the continual disturbance of the mud produces a substantial demand on the dissolved oxygen in the water of the upper estuary. The results, however, are not quite satisfactory in two respects: There was a very large oxygen uptake on first mixing the mud with the clean sea water, and the mud in the quiescent samples took rather long to settle initially. The test method was modified slightly in an attempt to overcome these difficulties, but in all cases the oxygen uptake from the shaken samples was greater than that from the quiescent samples. (Collett, 1961.)

CAN WE LIMIT BOTTOM DEPOSITS BY IMPOSING SUSPENDED-SOLIDS STANDARDS ON EFFLUENTS?

In assaying answers to the first four questions we have been able to look to factual evidence produced by field observation and laboratory investigation; we now come to the realm of opinion. It is in this region that the greatest potentiality for sincere disagreement arises, and it is here, perhaps, that the most valuable discussion can arise. The author will concentrate on the British viewpoint, confident that many in his audience will be particularly qualified to put the American view.

There will be little argument that there must be some control of the amount of suspended solids discharged to a stream or lake. Herbert (1961), for example, has shown that 1,000 ppm of china clay (an inert solid) considerably reduced the population of brown trout in Cornish streams, possibly owing to destruction of bottom fauna. In addition, a substantial proportion of the trout in the more turbid waters "showed a thickening and coarsening of the epithelial cells of their gills - a condition which has been observed in trout kept in suspensions of china clay and other inert material in the laboratory, but which was not found in control fish kept in clean water" (Herbert, 1961). Even 60 ppm of such inert suspended matter may affect spawning grounds but is unlikely to be harmful otherwise.

It is clear that bottom deposits will be produced, even in fast-flowing streams, by suspended-solids concentrations as great as we have just been discussing. Quite apart from possible contribution to oxygen demand, therefore, it is clear that suspended-solids standards must be set up to prevent such deposits. Such standards will normally result, after the appropriate dilution in the receiving water, in a relatively nonturbid stream, and it is the next stage of control that ought to concern us.

Even an effluent free from any suspended solids would, if it contained organic matter, produce, by bacterial synthesis, some suspended solids. So, although reasonably drawn suspended-solids standards can do much to limit bottom deposits, they cannot altogether eliminate them; for this we should require the complete removal from an effluent of organic matter as well. It is probably unrealistic, therefore, to consider suspended-solids standards in isolation.

WHAT RATIONAL CRITERIA CAN WE APPLY TO SUCH STANDARDS?

As Van Beneden (1957) emphasizes, we are too much concerned in our consideration of receiving waters with their self-purification capacity. As he says, we tend to take account only of the supernatant water, and we are hypnotized by its capacity for taking up oxygen from the atmosphere, forgetting that the body of water is acting also as a settling tank. In this review, we have been concentrating on this latter aspect.

The Royal Commission (1913) investigators, as we have seen, frequently emphasized the possible deleterious effects of bottom deposits and certainly took account of suspended solids in proposing their famous standards, which may be expressed in this form *:

1. Dry-weather dilution of effluent by river 500:1 or more - crude sewage may be discharged after screening and grit removal;
2. dry-weather dilution between 300:1 and 500:1 - sewage to be settled to give suspended-solids concentration of less than 150 ppm;
3. dry-weather dilution between 150:1 and 300:1 - sewage to be chemically coagulated and settled to give suspended-solids concentration of less than 60 ppm;
4. dry-weather dilution between 8:1 and 150:1 - sewage to be given full treatment to reduce suspended-solids concentration to less than 30 ppm and to give a 5-day (18° C) BOD of less than 20 ppm, including the BOD of any suspended solids;
5. dry-weather dilution less than 8:1 - specially stringent standards called for.

The Appendix to the Eighth Report goes on: "The quality of the diluting water would also have to be specified. Probably this would be fixed at about [2 ppm] of dissolved oxygen taken up from water in 5 days, no less dilution being allowed with water of greater purity. A water taking up [4 ppm] of dissolved oxygen in 5 days could not be considered as giving any dilution, since it is, itself, on the verge of causing nuisance; while a water taking up [3 ppm] must also be looked upon as of very doubtful quality as a diluent for unpurified sewage or sewage liquor." (Royal Commission on Sewage Disposal, 1913.)

These recommended standards were certainly based on the results of wide observation of a large number of English streams. It is possible, nevertheless, that these observations overestimated the effectiveness of the mixing that occurs below a sewer outfall. In the half century since the publication of this famous Appendix, the dry-weather flows of many important English rivers have been reduced by increased water abstraction and by improved land drainage, which has seriously reduced groundwater storage. It is not unlikely, therefore, that some important receiving waters have BOD's of more than 2 ppm and, therefore, give less effective dilution. Probably the Royal Commission's 20/30 standard should now demand dilutions of no less than 15 or 20:1.

With these dilutions available, the 20/30 standard should be adequate to prevent excessive buildup of sludge deposits, except immediately below the outfall. Where the velocity of flow, however, is very low, or where the water is quite still, more stringent standards are likely to be necessary.

Some of the rivers around London are in a peculiarly serious position: many of them are sources of potable water, their flows are very low, and they receive drainage from large concentrations of population. All these factors call for much more stringent effluent standards. The Rye Meads sewage treatment works, for example, has to achieve a 10/10 standard in winter and a 5/5 standard in summer. This calls not only for the highest standards of operation but also for tertiary treatment.

Indeed, there is a tendency for the English River Boards to demand that the suspended-solids concentration of sewage effluents be reduced substantially below 30 ppm. Some improvement can be achieved by increasing the capacity of the final settling tanks, but, aside from the cost, this is usually undesirable for settling following activated-sludge treatment. In Britain today a significant reduction in suspended solids, where it is called for, is usually obtained by microstraining or rapid sand filtration.

CONCLUSION

This review is intended to stimulate discussion, not to give an ex-cathedra pronouncement that stifles all discussion. Questions have been posed on the extent to which bottom deposits contribute to the oxygen demand in streams, and some observations have been culled from the literature to assist in answering these questions.

Most authorities concerned with the control of river pollution have, by implication, answered the various questions, but the author doubts whether the answers are, in most cases, any more than the codification of habit and prejudice. Practical action has, in this field, been based on inadequate field observation or not entirely relevant laboratory investigation. A good deal more work is required on the in situ measurement of oxygen uptake by bottom

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* In fact, standards nos. 1 to 3 were expressed the other way round, i e , the minimum dry weather flow dilutions were given for the specified treatments, which were expected to give the suspended-solids concentrations laid down.

muds. In addition a thorough investigation of the fate of suspended matter discharged to, or produced in, receiving waters is urgently needed.

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ACCUMULATION OF CESIUM-137 THROUGH THE AQUATIC FOOD WEB

Robert C. Pendleton *

The evaluation of the hazard to man of cesium-137 from fallout has been based on uptake from terrestrial food chains (Anderson *et al.*, 1957; Miller and Marinelli, 1956; Langham and Anderson, 1957; Marinelli and Rose, 1957; Libby, 1957; Langham and Anderson, 1959). It seems appropriate, therefore, to show the difference in bioaccumulation of this isotope in an aquatic environment and relate these data to the food web through which cesium may be accumulated by man. If, as Anderson and Gustafson (1962) report, cesium-137 may contribute as great a dose to human bone as strontium-90, ecological differences affecting the accumulation of cesium assume a greater importance than formerly was thought.

This report, a followup to a previous report (Pendleton and Hanson, 1958), contains results obtained in a study of the absorption and transfer of cesium-137 by organisms, water, and sediments in a small aquatic communtiy. Results of laboratory experiments

Table 1. ACCUMULATION OF Cs ^{137} BY AQUATIC PLANTS GROWN IN A SIMULATED FARM POND

Organism	Concentration factors [a]
Algae	
1. *Rhizoclonium crassipelitum* West and West	900 to 4,000 [b]
2. *Spirogyra crassa* Kuetzing	100 to 400
3. Mixed phytoplankton	1,200 to 14,000 [c]
Submerged seed plants	
1. Water weed (*Elodea canadensis* Michx.)	300 to 1,000
2. Coontail (*Ceratophyllum demersum* L.)	200 to 1,500
3. Sago pondweed (*Potomogeton pectinatus* L.)	600 to 1,000
Floating plants	
1. Water fern (*Azolla filiculoides* Lam.)	200 to 300
2. Duck weed (*Lemna minor* L.)	400 to 600
Emergent seed plants	
1. American bullrush (*Scirpus americanus* Pers.)	
Culms	50 to 100
Seeds	60 to 300
2. Hardstem bullrush (*Scirpus acutus* Muhl.)	
Culms	50 to 100
Seeds	200 to 400
3. Broadleaf cattail (*Typha latifolia* L.)	
Leaves	200 to 700
Seeds	100 to 700
Marsh plants	
1. Green bristle grass (*Chaetochloa viridis* Scribn.)	
Leaves	50 to 250
Seeds	80 to 270
2. Spotted persicaria (*Polygonum persicaria* L.)	
Leaves	200 to 700
Seeds	400 to
3. Marsh marigold (*Bidens tripartita* L.)	
Leaves	100
Seeds	300

[a] Concentration factor $= \frac{\text{Cs}^{137}/\text{g organism}}{\text{Cs}^{137}/\text{ml water}}$

[b] Contamination levels of Cs ^{137} in organisms varied with seasons and other environmental changes. The ranges of values shown are mean values for minimum and maximum uptake conditions. Single values indicate organisms sampled during only one season.

[c] A higher concentration factor for plankton (25,000) was observed on one sampling period.

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have been combined with reports of other workers to illustrate the effect of aquatic conditions on the accumulation of cesium-137 in the foods of man.

DIFFERENTIAL AVAILABILITY OF CESIUM TO AQUATIC AND TERRESTRIAL PLANTS.

Uptake of cesium-137 by terrestrial plants is low (Rediske and Hungate, 1955; Klechkovsky, 1957) because, upon entering most soils, cesium is quickly and firmly bound to the soil surfaces (Christenson *et al.*, 1958; Neel *et al.*, 1953; Nishita, Kowalewsky, and Larson, 1954) and is, thereafter, only slightly available to plants. Consequently, terrestrial plants have little chance to accumulate the isotope from soil. In contrast, aquatic plants exposed to cesium-137 in an aquatic ecosystem accumulated at least 500 times as much of the isotope as plants grown in soils, which shows a large difference in the availability of cesium-137 to aquatic food webs as compared with terrestrial food webs (Pendleton and Hanson, 1958). The high uptake values reported for some algae (Williams and Swanson, 1958) and for fish flesh (Knobf, 1951; Krumholz, 1956) agree with this interpretation. Klechkovsky (1957) reported a much higher uptake of this istope by cereal plants grown in nutrient culture than by plants grown in soil. The data in Table 1, which were obtained from plants grown in an aquatic community, indicate that if cesium can reach the root or other plant surface through a water continuum, it is taken up readily or adsorbed upon the plant surface, but at different levels according to species. Strong differences that correlate with ecological changes are also apparent.

The high contamination levels in aquatic plants, particularly submerged species, are thought to result from rapid sorption of cesium-137 before the ions can be fixed by other surfaces such as mud. This rapid sorption was shown by the rate at which algae accumulated the isotope after the first "spiking" of the pond. Floating rafts of *Rhizoclonium* took up cesium-137 so rapidly that 10 minutes after the isotope was added, survey instruments showed the rafts could be defined as radioactive "islands." No comparable means has been shown for the accumulation of cesium by terrestrial plants with the exception of a limited amount of foliar absorption and entrapment of particles on aerial surfaces.

Many emergent plants inhabiting saturated soil or shallow water develop root systems at or above the soil level. Such superficial root systems lie in an area from which cesium ions may be readily obtained. Large increases in uptake of cesium-137 by plants grown in shallow water and saturated soil as compared with upland habitats have been demonstrated (Pendleton and Uhler, 1960; Tensho, Yeh, and Mitsui, 1961).

Thus, the root ecology of wetlands contributes to increased availability of cesium-137 to aquatic food webs by adding higher root absorption to the amount from direct fallout upon the plant surfaces. The evidence presented indicates that, given equal contamination, the availability of cesium-137 is markedly greater from aquatic situations than from the terrestrial environment. Further evidence that accessibility of the ions is important appears in Table 2 where the difference in accumulation of cesium-137 by plants rooted in sand and gravel as contrasted with those grown in mud is shown.

AVAILABILITY OF CESIUM TO AQUATIC ANIMALS

Because of the increased availability of cesium to aquatic plants, aquatic animals also show high concentration factors for this element (Table 3).

In the concentrations shown by different trophic levels, another factor influencing the accumulation of cesium is illustrated. This factor is of sufficient importance to warrant examination of the evidence supporting it, and is also an attempt to explain the biological reasons for its occurrence.

ACCUMULATION OF CESIUM-137 ACCORDING TO TROPHIC LEVELS

With the exceptions of some species of algae that had higher values, insect predators that had lower values, and several species that had some seasonal high values, contamination levels of cesium-137 increased directly with the trophic level (Tables 1 and 3). Trophic-level dependence for cesium-137 accumulation has been reported before (Pendleton and Hanson, 1958; Pendleton, 1959), and field studies

Table 2. INFLUENCE OF SUBSTRATE ON ACCUMULATION OF Cs ^{137} BY EMERGENT PLANTS

Organism	Concentration factors					
	Grown in mud			Grown in sand and gravel		
	Leaves	Roots	Seeds	Leaves	Roots	Seeds
Cattail	70	170	100	250	250	100
	Culms			Culms		
Hardstem Bullrush	50	40	90	120	450	300

Table 3. ACCUMULATION OF Cs^{137} BY ANIMALS GROWN IN A SIMULATED FARM POND

Organism	Concentration factor
Herbivorous animals	
1. Water snail (*Radix japonica* Jay)	
Entire	600
2. Bullfrog tadpole (*Rena catesbeiana* Shaw)	
Entire	2,600
Gut and contents	4,500
Flesh	1,000
3. Spadefoot toad tadpole (*Scaphiopus hammondi intermontanus*)	
Entire	6,000
Carnivorous animals	
1. Damselfly nymphs (*Ischnura* sp.)	
Entire	500 to 800[a]
2. Dragonfly nymphs (*Erythemis callocata* Hag.)	
Entire	400 to 800
3. Pumpkinseed (*Lepomis gibbosus* L.)	
Adult, entire	2,000 to 7,500
Muscle	7,000 to 11,000
4. White crappie (*Pomoxis annularis* Raf.)	
Entire	2,000
5. Bluegill (*Lepomis m. machrochirus* Raf.)	
Entire	6,500
6. Bullfrog (*Rana catesbeiana* Shaw)	
Muscle	8,000 to 11,000
Omnivorous animals	
1. Carp (*Cyprinus carpio* L.)	
Entire	1,000
Muscle	2,000 to 3,000
2. Water shrimp (*Hyalella azteca* Saus.)	
Entire	1,400 to 11,000

[a] Ranges of values are means for minimum and maximum uptake. Variations stem from seasonal and other ecologic differences. Single values indicate sampling during one season only.

have shown trophic level effects on accumulation of cesium-137 through the situation was not recognized (Hanson and Browning, 1956; Hanson and Kornberg, (1956).

The effect of trophic levels on contamination in animals is more fully shown in Table 4. These organisms were sampled on the same day at the end of the pond experiment in their entirety, and the cesium levels were determined with a gammaray spectrometer.

The omnivore, as represented by carp or man, reflects the multiple sources of food and has contamination levels lower than those of predators, such as sunfish or frogs.

The pumpkinseed and crappie are primary predators, feeding mainly upon insects and crustaceans, although some vegetation may be taken (Simon, 1946). The normal diet of the small bluegill is not greatly different from that of the pumpkinseed and crappie, but at the time these three species were compared, the bluegills (all large adults) were feeding on young sunfish and thus assuming the role of secondary predator.

The high accumulation in the higher trophic levels apparently stems from two metabolic characteristics of cesium: high gut absorption and biological half-life (Tb 1/2) relative to potassium. Ingested cesium is 50 to 100 percent absorbed from the gut by the animal (Ballou and Thompson, 1958; Richmond, 1958; Ilin and Moskalev, 1957; McClellan *et al*, 1959); much of the total intake, whether adsorbed upon the surface or incorporated in the cells of the food, is transferred to tissues of the consumer.

The trophic-level increase, although different from results obtained with other isotopes (Davis and Foster, 1958; Donaldson, 1954), is consistent with the peculiarities of accumulation and retention of cesium and potassium. For example, the Tb 1/2 for cesium is 13 days in the rat (Ballou and Thompson, 1958), 25 days in the dog, and 110 to 150 days in man (Richmond, 1958); the Tb 1/2 for potassium in the same three organisms is 4, 8, and 58 days (Richmond,

Table 4. TROPHIC LEVEL EFFECT ON THE CONTENT OF Cs ^{137} IN FOUR SPECIES OF FISH TAKEN FROM A CONTAMINATED POND

Species	Trophic level	Cs ^{137}, μc/g [a]	Concentration factors
Carp (*Cyprinus carpio* L.)	2,3,4 (Omnivore)	3×10^{-3} [b]	1,000
Pumpkinseed (*Lepomis gibbosus* L.)	3 (Primary predator)	6×10^{-3}	2,000
White crappie (*Pomoxis annularis* Raf.)	3 (Primary predator)	6×10^{-3}	2,000
Bluegill (*Lepomis m. macrochirus* Raf.)	4 (Secondary predator) [c]	2×10^{-2}	6,500

[a] Contamination level in water = 3×10^{-6} μc/ml.

[b] Contaminants (Zm-^{65} and Zr-^{95} - Nb ^{95}) approximately equal to 1% of the Cs ^{137} were found in all fish.

[c] Stomachs of these fish contained only immature pumpkinseed when sampled.

1958). Thus, cesium remains in these organisms at least three times as long as does potassium. It is probable that the same relationship between the Tb 1/2 of cesium and potassium exists in other organisms.

When available in sufficient quantity, cesium competes with potassium and may replace more than half the latter element in the muscle of frogs and rats (Lubin and Schneider, 1957; Relman *et al.*, 1957). If this condition is general, it is probable that contamination levels of cesium-137 in animals are controlled only by the availability of cesium in the food supply, and hazardous concentrations may be expected if gross contamination occurs. This is further indicated by a one-step food chain experiment in which algae accumulated cesium-137 to 2.2 microcuries per gram, and flesh of tadpoles feeding on the algae accumulated 6.8 microcuries per gram before cessation of feeding at metamorphosis.

Although changes in dietary potassium have little effect on accumulation or retention of cesium-137 by animals (Wasserman and Comar, 1961), it is probable that the two ions share the same transport mechanism, and this factor may partially explain the differences in Tb 1/2. A possible mechanism is shown in Figure 1.

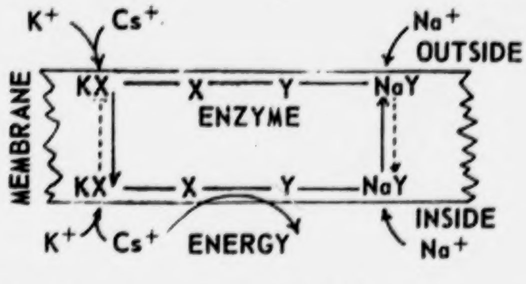


Figure 1. "Hypothetical scheme of a Na^+ - K^+ exchange pump. The substances X and Y are assumed to be confined to the membrane. X has a high affinity for K^+; Y has a high affinity for Na^+. X and Y move through the membrane only when in combination with an ion." Diffusion of Na^+ into and K^+ or Cs^+ out of the cell is shown with dotted lines. (Adapted from Woodbury, 1960).

If cesium enters the cell in combination with X, and the cesium competes inefficiently with potassium for sites on the X molecule, the enzyme that removes potassium from X may not have the energy or configuration to remove cesium efficiently from the X substance. Once freed, the cesium may have a slower passive diffusion rate or form complexes with the cell proteins, as suggested by Wasserman and Comar (1961), further reducing the rate of egress. This model fits the known differences in accumulation of cesium and potassium (slower uptake of cesium and slower outward diffusion), but does not explain the threefold difference in Tb 1/2 that has been noted. The combination of metabolic factors presented above for cesium and potassium, however, shows that the increased concentration factors for cesium-137 in successive trophic levels can be explained on this basis, and the organism must come to equilibrium with its integrated food base at a contamination level higher than that of its food. Observed threefold increases are further proof of this relationship. The buildup to a new contamination level has been described by Anderson, Schuch, Fisher, and Langham (1957) who showed that a stepwise increase in the cesium-137 content of food is followed by an adjustment by the consumer at a higher level, theoretically 3.4 times higher than the new contamination level in the food. Richmond, Furchner, and Trafton (1962) showed whole-body retention of cesium-137 in mice to be near 300 percent of the daily intake, and the same relationship of food level to whole-body burden has been demonstrated with rams by McClellan, McKenney, and Bustad (1959). Marinelli and Rose (1957) showed that in humans the slower excretion rate of cesium relative to potassium leads to a cesium/potassium ratio of from two to three times the ratio in the food and that Oriental people contain smaller amounts of cesium-137 than people in the United States who consume more meat and dairy products. This difference emphasizes the effect of a shift from a predominantly herbivorous diet to a food base more like that of a predator. This may partially explain why populations such as the Swedish Laplanders (Andersson, and Nilsson, 1961) who rely heavily on animal products for food have high body burdens of cesium-137 One might predict that high body burdens would be found in the Eskimo and Indian populations of interior Alaska and Canada, and Massai of Africa. The environment of the interior Eskimo and Indian populations is a high cesium-137 accumulation area similar to that of the Swedish Laplanders, but, essentially, the reason would be because carnivorous diets should produce high cesium-137 body burdens compared with omnivorous or herbivorous diets. Conversely, lower levels of cesium-137 should be expected in people who obtain much of their calories from refined sugar and oils--both low in cesium-137 content.

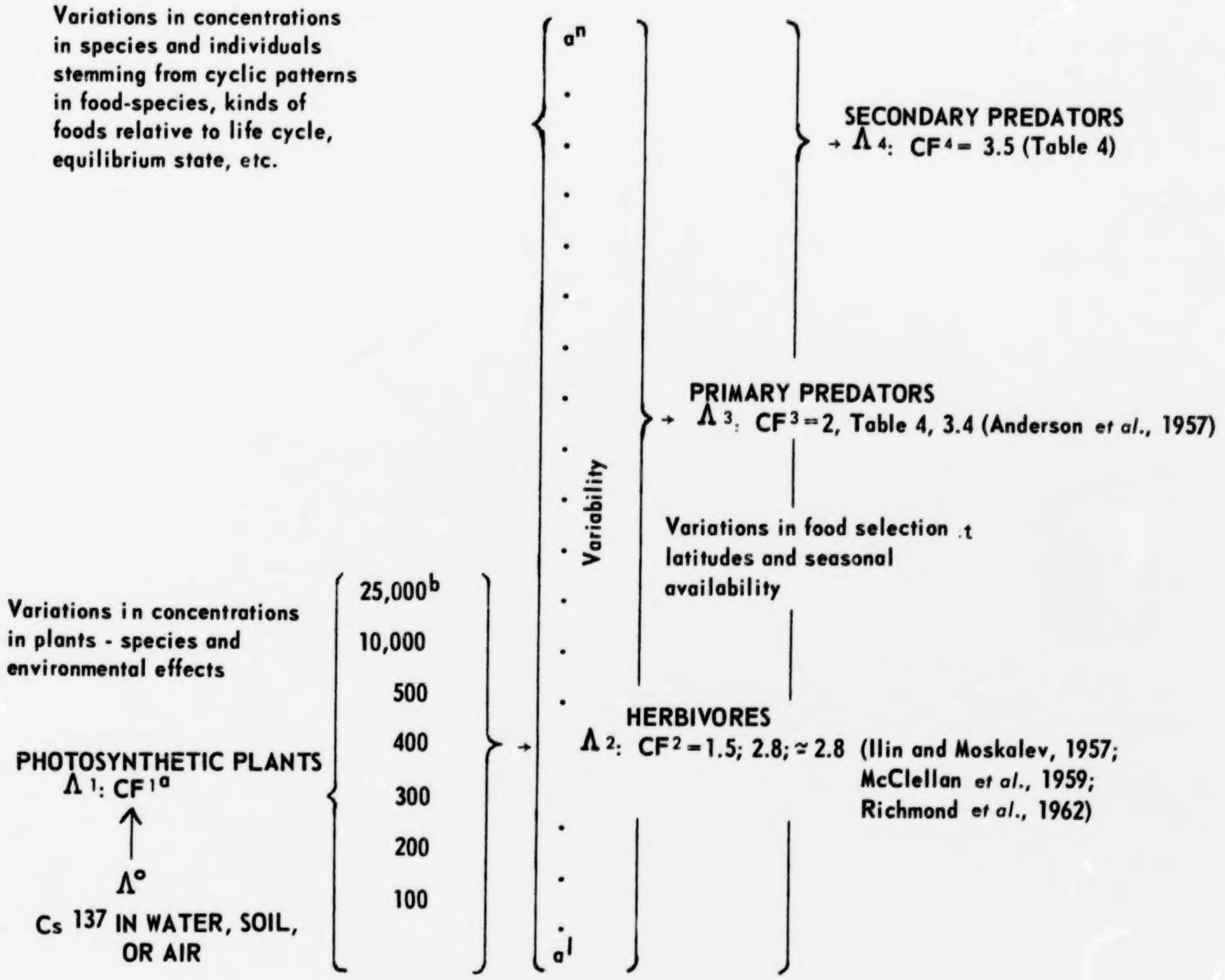
Each successive trophic level represents a good base for the organism that feeds upon it, with the same result as a seasonal or annual contamination increase. The relationship of trophic level to concentration is better illustrated diagrammatically:

$$\left(\begin{Bmatrix}\text{Water}\,\Lambda^{0}\\ \text{Cs 137 in Sol.}\\ 3\times10^{-6}\,\mu c/ml\end{Bmatrix} \rightarrow \begin{Bmatrix}\text{Algae}\ \Lambda^{1}\\ CF = 1{,}000^{a}\\ 3\times10^{-3}\,\mu c/g\end{Bmatrix} \rightarrow \begin{Bmatrix}\text{Herbivore}\,\Lambda^{2}\\ CF = 3\\ 9\times10^{-3}\,\mu c/g\end{Bmatrix} \rightarrow \begin{Bmatrix}\text{Primary predator}\,\Lambda^{3}\\ CF = 3\\ 2.7\times10^{-2}\,\mu c/g\end{Bmatrix}\right)$$

[a] Arbitrary concentration factor based upon Table 1.

The contamination level in a predatory fish of 2.7 x 10^{-2} μc/g represents a contamination factor of 9,000. This is in agreement with concentration factors for muscle from Λ^3 predators grown in water contaminated to 3 x 10^{-6} μc/ml in the pond experiment but is higher than the maximum levels in entire sunfish.

In the aquatic community, observed increases between trophic levels were usually less than the expected threefold increase in contamination. This difference becomes clearer when related to the variability inherent in food webs. The food species consumed by an herbivore or predator may be numerous, and kinds and numbers may change with age, seasons, and many other factors. Also, the predator may consume organisms from several trophic levels. Because the many species consumed by the herbivore


Variations in concentrations in species and individuals stemming from cyclic patterns in food-species, kinds of foods relative to life cycle, equilibrium state, etc.
a^n
SECONDARY PREDATORS
→ Λ_4: CF^4 = 3.5 (Table 4)
PRIMARY PREDATORS
→ Λ_3: CF^3 = 2, Table 4, 3.4 (Anderson et al., 1957)
Variability
Variations in food selection at latitudes and seasonal availability
$25{,}000^b$
10,000
500
400
300
200
100
Variations in concentrations in plants - species and environmental effects
PHOTOSYNTHETIC PLANTS
Λ_1: CF^{1a}
Λ^o
Cs 137 IN WATER, SOIL, OR AIR
HERBIVORES
Λ_2: CF^2 = 1.5; 2.8; ≈ 2.8 (Ilin and Moskalev, 1957; McClellan et al., 1959; Richmond et al., 1962)
a^1

[a] CF^1 = Concentration Factor. $CF = \dfrac{Cs^{137}\text{ in plant}}{Cs^{137}\text{ in}\Lambda^{o}}$

[b] Numbers represent known differences in concentration factors.

Figure 2. Factors affecting variation in accumulation of cesium-137 by organisms.

may each have a different contamination level, the body burden of cesium-137 in the consumer represents an integration of all its food sources (Figure 2). Consequently, a clear-cut threefold increase between trophic levels should not be expected to occur often in nature at contamination levels from fallout, and the presence of highly contaminated plants or herbivorous species does not constitute an exception to trophic level increases. Such organisms may comprise either a large or small fraction of the food of a consumer.

In the pond study, the theoretical threefold increase in contamination from prey to predator was well defined in only one case, i.e., juvenile sunfish (2,000) to bluegill adult (6,500) (Tables 3, 4), and in this instance the predator was feeding almost exclusively on the one species. Invertebrate herbivores, plankton feeders, and predators (Table 4) showed less contamination than many of the organisms on which they feed. This apparent reduction resulted from the sampling methods. The insects, crustaceans, and mollusks were sampled entire, and the concentration per gram is based upon the whole weight, which includes ceolomic fluids, exoskeletons, and shells. This sampling method, as shown by Zhadin, Kuznetsov, and Timofeev-Resovsky (1958), gives a concentration factor for the entire animal that is much lower than that of the body sans shell. The vertebrate predator, feeding upon these organisms, obtains cesium at the level of the concentration in soft parts and voids the water, chitin, and calcareous shell.

DISCUSSION

Because there is a difference in availability of cesium-137 to plants and animals in an aquatic environment compared with a terrestrial site, meat and dairy products produced where a large proportion of the forage is from wetlands should contain more of this contaminant than products from drylands. This may be one factor causing the observed variation in cesium-137 content of milk from different locations of similar rainfall (Anderson, 1958; Booker, 1957), for it is probable that uptake of cesium from fallout by grasses growing in saturated or flooded meadows will be similar to that of the grass (Green Bristle Grass, Table 1) rooted in saturated mud in this experiment. Similarly, crops grown in saturated soils or standing water should contain more cesium-137 than crops grown in drier soils, as shown by Tensho, *et al.* (1961). Further, a few dairy herds might contribute disproportionately to the cesium-137 content of a local milk supply, and extensive wetlands could elevate the mean contamination level of a larger region. If cesium-137 contamination in milk should ever approach hazardous levels, some reduction might be possible by preventing dissemination of dairy products from areas in which high accumulation is known to occur, or by moving the herds to drier areas.

The relative hazard of cesium-137 accumulation through an aquatic versus a terrestrial environment may also be illustrated by comparing fish-farming with grazing.

The annual yield of edible meat products from improved grazing land is about 150 to 700 kilograms per hectare, and a hectare of fertilized pond may provide 300 to 3,000 kilograms of fish (Clarke, 1954; Odum, 1959). By arbitrarily assuming a mean concentration factor of 1,000 for the flesh of the fish crop the potential yield of cesium-137 to man is manyfold greater per hectare than from a comparable unit of grazing land, because the flesh of cattle should not contain more than three times the cesium level in the forage consumed. The contamination level of the terrestrial forage would, under drylands conditions, be essentially the same as the amount from direct fallout less the amount fixed in the soil.

Similarly, a comparison of the yield per hectare of fish of three different trophic levels is of interest. Clarke (1954), has shown that goldfish (*Carassius auratus* (L.), which are mainly herbivores, may yield 500 kg/ha/yr, but bluegill, a primary carnivore, yields 250 kg/ha/yr and bass, a secondary carnivore, yields only 100 kg/ha/yr. The productivity ratio of these three fish is 5:2.5:1. In the pond experiment, carp, sunfish, and bluefill represented the three trophic levels described by Clarke. It is of interest that the concentration factors for the three trophic levels make a ratio of 1:2:6.5 and, when these values are multiplied by the productivity ratio, the products are quite similar. This may indicate that the levels of cesium-137 in trophic levels of an aquatic site may be useful to determine the relative balance of the unit as well as the degree of predation and probable foods of individual species.

Trophic level	Concentration factor ratio		Productivity ratio		Product
2	1	x	5	=	5
3	2	x	2.5	=	5
4	6.5	x	1	=	6.5

One might infer that under balanced conditions, the amount of cesium-137 in the standing crop of fish will be divided somewhat equally among the three trophic levels, but, because the higher trophic levels obtain the same or slightly greater amounts of cesium-137 from fewer kilograms of fish, the hazard relative to food for man would increase with the trophic level. This effect may be augmented by the preference for food of fish from the higher trophic levels. The low yield of predaceous fish from most natural fresh waters, however, would not contribute much cesium-137 to local populations unless a high proportion of the food was from this source.

The effects of high accumulation in the higher trophic levels are also of interest in regard to the maximum permissible concentration (MPC) for cesium-137 in drinking water of 4 x 10^{-4} microcuries per milliliter (NBS Handbook 69, 1959). Simple calculations show that flesh from a fish grown in water contaminated to the MPC will become much too contaminated for use as human food. For example, flesh of the pumpkinseen accumulated cesium-137 to 3 x 10^{-2} microcuries per gram weight from foods grown in water containing 3 x 10^{-6} microcuries per milliliter. Only 2.2 pounds of this flesh would supply 30 microcuries of cesium-137, a quantity equal to the long-term, maximum permissible whole-body burden for this isotope. If similar increases occurred at the MPC level for water, the predator could accumulate 3.6 microcuries per gram, and less than 10 grams would contain 30 microcuries. Although the MPC for drinking water was not intended as a criterion for disposal and the use of the 40-hour level is questionable, having been used for illustration only, the above comparison suggests that disposal to fresh water should be kept at least 10^{-3} less than the MPC for drinking water. In the absence of information, however, about accumulation of cesium-137 by organisms in many, different aquatic communities, advancing an "ecological MPC" for this isotope is not justified.

The basic differences in the availability of cesium-137 to the foods of man from aquatic as compared with terrestrial sources are summarized in Table 5.

From the comparison of availability of cesium-137 to foods through the aquatic and terrestrial food routes, it may be inferred that, although the major portion of cesium-137 in the world's population will come from terrestrial sources, the probability of accumulation of hazardous quantities is much greater from aquatic or wetland environments.

Differences in ecological conditions cause great variation in accumulation of cesium-137 in organisms, and the differences ascribable to water abundance exceed those from soil chemistry or type by many orders of magnitude (Fowler and Christenson, 1959; Pendleton and Uhler, 1960). These variations suggest that evaluation of hazards from cesium-137 in fallout should be made on local, rather than a region-wide, basis since populations with specialized food habits that live in an area where high absorption occurs could be receiving much more radiation than the regional mean. Trophic level increases of approximately threefold add to the problem.

Cesium is apparently not accumulated in marine environments to the same degree as in fresh water (Revelle and Schaefer, 1958); hence, high concentrations of cesium-137 should not be expected in foods from the ocean. Also, in large bodies of fresh water such as rivers and lakes where dilution is great, lesser hazards should accrue, although eviscerated trout from streams and lakes in Utah (Sept. 1962) contained 700 picocuries of cesium-137 per kilogram while flesh of mule deer, feeding in the same area on plants heavily contaminated by fallout, contained about the same level (Pendleton, Unpublished manuscript). Dilution in these aquatic situations was great as compared with the terrestrial condition.

Table 5. COMPARISON OF FACTORS THAT AFFECT CONTAMINATION LEVELS OF Cs ^{137} IN THE FOODS OF MAN

Aquatic food route	Terrestrial food route
1. Reduction by dilution and adsorption on mud.	1. Reduction by adsorption on soil particles.
2. Rapid absorption by algae and other plants, increased root absorption, plus external adsorption or entrapment.	2. Limited foliar absorption, low root absorption, plus external adsorption or entrapment.
3. Concentration factor $>$50.	3. Concentration factor $<$1.
4. Level in plants greater than environmental contamination.	4. Level in plants less than or equal to the level of environmental contamination.
5. Two to four trophic levels available to man. Man feeds upon 2, 3, or 4.	5. Normally not more than three trophic levels available to man. Man feeds on 1 or 2.

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DISCUSSION

(Paul O. Fromm)

Dr. Fromm's paper was presented and discussed first. It was followed by Dr. Alderdice's paper and further discussion. Dr. F.E.J. Fry, who was asked to make some general comments by way of summary, noted that Dr. Fromm is seeking some sensitive measure or index of chronic intoxication that is largely divorced from the result of primary concern, namely, eventual damage to the organism. Dr. Alderdice's objective, it was pointed out, is very different. The system of analysis and the models presented and explained by him will facilitate consideration of the toxicity (demonstrably harmful action) of a water pollutant under a wide variety of circumstances or combinations of environmental conditions that may occur in nature. The interaction of environmental variables or factors that may jointly influence the toxicity in otherwise unforeseeable ways can thereby be effectively investigated. Among the questions raised in the course of the informal discussions were the following:

(1) Can a substance whose action is not demonstrably fatal or incapacitating be referred to properly as a toxic substance, and what is the correct distinction, if there is any, between "chronic" and "subtle" effects of a toxicant or water pollutant? The consensus of opinion seemed to be that a substance that is not acutely toxic and even one that is incapable of incapacitating an organism or causing its death after prolonged exposure can nevertheless be said correctly to be a toxic substance. Chronic intoxication kills or incapacitates only after a long time or period of exposure. Subtle effects of toxicants may be such effects as a reduction of the exposed subject's appetite (food intake) or some deformity of young produced by exposed adults who are not themselves demonstrably affected.

(2) Is not the assumption that histological, biochemical, or other evidence of changes in the tissues or cells of aquatic animals exposed to water contaminants indicates significant or incipient injury to the organisms and to their productivity often an unjustified assumption? In other words, before much practical significance can be attached to such evidence, is it not necessary clearly to demonstrate that the observed responses are not inconsequential or merely adaptational and that they are indeed accompanied or followed by some related impairment of performance or of functions essential to the continued well-being and undiminished productivity of the animals? This question was not fully answered. It was noted that biochemical and histopathological studies often explain previously observed harmful effects of toxicants. However, the statement was made also that the manner of action of toxicants usually is not really known or fully understood. This observation suggests that the effects of toxicants of the cellular or tissue level (biochemical, etc.) that have been reported and measured are not often effects that have been shown definitely to be directly related to the lethal action of the toxicants or to any observed functional impairment at sublethal toxicant concentrations. The need for establishing a direct connection and quantifying the relation between the former effects and effects on the performance of the organism as a whole seems to have been too often neglected. The comment was made that fish toxicologists are far ("90 years"!) behind mammalian toxicologists, who certainly have given more attention than have fish toxicologists to the evaluation and explanation of the subtle effects of sublethal levels of intoxication on performance, behavior, etc. However, no evidence was brought forth to show that mammalian toxicologists have already achieved great understanding or knowledge of the relations between these subtle effects and the observed biochemical or other changes in mammalian tissues, and so are

not any longer faced, in large degree, with problems such as those considered above with particular reference to fish and other aquatic animals.

(3) What can be done to promote intensified biochemical, physiological, and pathological research relating to problems of water pollution and its effects on aquatic life? It was pointed out that biochemists, pathologists, etc., now have plenty of work to do without becoming involved in problems of fish toxicology and they must be somehow attracted to work in this field. Better financial support for the studies in question appears to be the key to the solution of this problem. However, it is much easier to obtain financial support for applied research of evident and immediate practical value than for more basic research, and for this reason applied research, moving ahead of basic research, is often seriously hampered by a dearth of essential basic data and principles. To ensure much better support for the needed basic biochemical, physiological, and other studies, the practical utility of data or knowledge obtained through such studies (i.e., the efficiency of the approaches to the solution of practical problems that are based on these findings) must be clearly demonstrated, as suggested earlier (see item 2).

(4) Dr. Alderdice was asked whether the model that he had presented and discussed in explaining his system of experimental design and analysis of bioassay results was intended to serve predictive purposes or to serve only as a basis for the planning of additional tests. He replied that he expects that it will have demonstrable predictive value or utility.

(Charles G. Wilber)

In using the organ to body weight ratio, care must be exercised to account for the normal physiological changes of the organs as, for example, the storage role of the liver. By comparison with the control group, however, these changes can often be overcome.

The suggestion was made that any "measurable" concentration of copper is deleterious. After some discussion of what is meant by "measurable," the question was answered on theoretical grounds that copper is a necessary trace element, and, therefore, there must be some concentration that would not be considered harmful.

(Behavioral Responses of Fish to Toxicants)

Mr. Richard Warner presented unpublished toxicity data on fish exposed to toxaphene for 24 hours and subsequently rested in fresh water 24 hours before testing. He used as the endpoint, the ability of the fish to acquire a conditioned response. Fish exposed to concentrations as low as 0.064 ppb evidenced marked differences from the controls. He closed his presentation by asking how such data can be extrapolated to the natural situation.

As a whole, the group was impressed with the sensitivity of this test and felt that such experiments would be very helpful in evaluating the sub-lethal effects of toxicants. The only answer that could be given to the question, however, was that we can only accumulate data of similar nature, and after enough is obtained, then perhaps it can be fitted together to give a nearly complete understanding of the effects.

Further discussion revealed a feeling by some that the choice of the above-mentioned type of approach, or the approach using more gross effects such as death and growth, depends upon the time one may have available to determine acceptable concentrations. Although the use of such sensitive tests might reveal more closely the true level of no deleterious effect, it may be necessary to use more direct approaches to set an approximate safe level when time is very short. The approximate levels, however, should be revised in light of more refined research.

Representatives from England pointed out that their philosophy is to set maximum permitted concentrations based on a safe level for three months. A three-month interval is used because it corresponds to the period of low flow, characteristic of their streams. They also consider that there is a minimum level that is always present, regardless of flow, and this level must also be safe for extended periods.

One concept was crystal clear from this discussion as well as from the conference, namely, that a colossal amount of work must be done with sub-lethal concentrations before sound judgement can be made as to what levels of toxicants are really safe.

(John B. Sprague)

Observations were reported that indicate that small concentrations of copper do cause some aquatic animals to increase their activity. Many unexplainable points concerning the pattern evidenced by Dr. Sprague's data were discussed, but no firm conclusions were reached.

(Comments on Dr. Sprague's paper
by Charles B. Wurtz)

Dr. Sprague introduced his comments on salmon avoidance of heavy metals with a slide showing curves for the movement of salmon and the amount of rainfall for the year 1956. These data were grouped in 5-day units. He did not mention that the salmon-counting fence was washed out by high flows for short periods in 1956, and, therefore, an accurate count of salmon was not possible. The inaccuracy of the salmon count would be aggravated by the upstream surges of salmon that are a product of freshet flows.

The second slide showed the 1960 upstream-downstream salmon movement in association with a curve for zinc concentration. No values were expressed for the zinc concentrations, but the correlation between higher zinc loadings and increased downstream salmon movements was not clearcut.

No apparent attempt was made to correlate the downstream movement with any other factor. And, once again, the salmon-counting fence was washed out by high waters for that year, rendering an accurate count of fish impossible.

The fourth slide shown correlated the 1961 salmon movements with the "toxicity index" method developed by R. Lloyd (Stevenage, England). The correlation with this very useful concept of Lloyd's was much closer than the correlation with the direct measure of zinc. However, the basic data from which the curves were developed are open to question. The "toxicity index" is a function of three variables: Zinc concentration, copper concentration, and hardness of water. Upon query, Dr. Sprague advised the group that daily analyses had been performed for zinc and hardness, but that copper was only measured once a week. In addition, although not brought forth during the discussion, the source of the samples analyzed differed. These samples were drawn from two locations that are 6.4 river miles apart. Dr. Sprague believes these sampling points to be virtually the same in water quality characteristics. This is most dubious. The lack of exact counts of the upstream-downstream movements of salmon, as mentioned above, would further depreciate the value of the curves as shown.

(Answers to comments by Dr. Wurtz on paper by J.B. Sprague.)

As Dr. Wurtz points out, it is unfortunate that the salmon-counting fence was not flood-proof. A histogram of salmon movements during 1956 was shown at the meeting to illustrate the usual movements of fish before the mine was active. For the period from June 5 to November 13, 1956, shown in the slide, the fence was inoperative only once, from June 15 to June 18. Of the salmon movements recorded that year, 2% of those going upstream came back downstream.

In 1960, the counting fence was inoperative for 3 days in late June, 3 days in late October, and was partially inoperative for one additional day in late October. These periods were indicated in the slide shown at the meeting, and in Figure 1. Similar inoperative periods in 1961 are shown in Figure 2.

During the winters, samples were taken 6.4 miles upstream of the counting fence, but all samples included in this paper were taken at the counting fence. The concentrations of copper in the Miramichi were very low, and difficult to analyse accurately. Accordingly they were calculated for 1961, for measurements of copper in the polluted tributary. This was done on the basis of the dilution ratios indicated by the concentrations of zinc at the same two locations. Refinements of analytical technique in 1962 show that this gave valid estimates. Sometimes the estimates of copper in 1961 were available only once a week. However, as shown in Figure 2, they were usually more frequent, as often as once a day. Suggestions for alternative explanations of the unusual disturbed migration would be welcomed by the author.

(Peter C. G. Isaac)

A discussion followed in regard to the oxygen demand resulting from the respiratory activities of benthic organisms. The general opinion was that the bottom organisms do, in fact, contribute greatly to the oxygen demand of bottom muds.

Dr. Gunderson described in brief a pollutional survey that was carried out on the Sacramento River, a relatively unpolluted stream. A point of interest was the fact that while some sections of the river had the classical diurnal oxygen curve, other sections revealed a totally inverted oxygen curve; that is, the oxygen levels rose during the night. Although there was no suitable explanation for this phenomenon, a suggestion was made that the variation in the respiratory activity of the bottom organisms might account for it. It was also suggested that the higher oxygen levels at night might be due to the presence of a chemical source of oxygen, such as nitrates, to the release of oxygen bubbles from the steams and leaves of higher plants, or perhaps to "moonlight photosynthesis."

Dr. Ohle pointed out that the oxygen demand of bottom muds and sediments is often increased by the presence of gases, especially methane. In addition to the oxygen necessary to oxidize these gases, some bacteria use these gases as a substrate, thereby further increasing the oxygen demand.

In discussing the standards for suspended solids, it was brought out that the allowable levels of suspended solids should depend upon the receiving waters, and, therefore, should vary.

(Dr. F. E. J. Fry)

The discussion following Dr. Fry's presentation ranged over a wide variety of subjects:

(1) Anaerobic metabolism in fishes. There is evidence that anaerobic metabolism in fishes can be important under some conditions, but it is difficult to measure, and consequently the extent of its possible influence, under different circumstances, upon the reliability of metabolic rate determinations based on evaluations of oxygen consumption rates is not yet clear.

(2) Influence of D.O. and free CO_2 on fish embryos and larvae. Available data indicate that the sensitivity of fish to reduction of dissolved oxygen is greatest just before hatching and decreases sharply thereafter. There is very little information available on the influence of free carbon dioxide on the dissolved oxygen requirements of larvae and embryos at different stages of development.

(3) Influence of water velocity on embryonic development of fish. There was no disagreement with regard to the expressed opinion that the flow of water past fish embryos probably influences their development (developmental rate, size of fry at hatching, etc.) chiefly by determining the rate of delivery of oxygen to the chorion surfaces and not the rate of removal of metabolites, but more information on this subject is needed.

(4) Dependence of oxygen uptake rates of aquatic invertebrates on oxygen concentration and water velocity. The probable significance was discussed of the frequently reported observations of dependence of the oxygen consumption rates of supposedly resting and fasting invertebrate animals (e.g., aquatic insects, etc.) upon oxygen concentration and water velocity even at high dissolved oxygen and water velocity levels far above those necessary for continued survival of the animals. One explanation that has been suggested is that the measured metabolic rates of the animals in question actually were far above the true resting levels (i.e., were "active" metabolic rates), because of the existence of an "oxygen debt," some persistent activity, or invisible muscular tension, etc., resulting from recent handling or other disturbance of the animals or evoked by unnatural environmental conditions. It is known that the oxygen uptake rate of fish may remain far above the standard rate and dependent on oxygen concentration at rather high dissolved oxygen levels for a number of hours after introduction of the fish into a respirometer. However, K.H. Mann presented some data on the oxygen uptake rates of different leeches showing dependence of the rates on oxygen concentration (at high as well as low D.O. levels) that did not seem to be readily explainable in the manner suggested above. The suggestion that a great depression of oxygen uptake rates at reduced oxygen concentrations may be due to suppression of metabolic processes involving translocation of body substance (i.e., "growth" at the expense of stored energy sources such as fat deposits) was considered, but it was rejected as an erroneous or unlikely explanation of the phenomenon under consideration, except in the case of a growing embryo or larva that derives nourishment from yolk. Finally, the suggestion was considered and generally accepted that a progressive increase of the ratio of anaerobic metabolism to aerobic metabolism resulting from a progressive decrease of oxygen availability could account for an observed decrease of the rate of oxygen uptake. No objection was raised to the view that, if life can be sustained increasingly by anaerobic metabolism as the dissolved oxygen concentration falls, a metabolic rate far above the basal rate (true resting level) at high oxygen concentrations need not be postulated to explain the ability of an animal to tolerate for long periods lower oxygen concentrations at which the oxygen uptake rate is greatly reduced. It was not possible to decide which one of the suggested, plausible explanations of the phenomenon under consideration is more generally appropriate or correct.

(5) Influence of the dissolved salt content and hardness of water on fish and on their resistance to disease and to temperature extremes. S. F. Snieszko reported that fish appeared to be more susceptible to kidney disease in soft water than in harder water. Fish kept in demineralized water for a week survived and suffered no apparent osmotic shock on return to their normal medium (fresh water). F.E.J. Fry stated that water hardness seems to influence the thermal tolerance of fish; in soft water they are apparently more tolerant of high temperatures than in harder water. P. Doudoroff remarked that secondary chill coma and death of marine and fresh-water fish that are exposed to lethal low temperatures can be delayed but probably cannot be prevented entirely by adjusting the salinity of the medium so as to render it approximately isosmotic with the body fluids and thus prevent the lethal effects of osmo-regulatory failure that occurs at the low temperatures.

(6) Evaluation of activity and metabolic rates of fish in the laboratory and under natural conditions. Devices for the measurement of spontaneous activity of fish in the laboratory, as related to rates of oxygen consumption, etc., were described and discussed. The difficult problem of how to estimate metabolic rates of normally active fish in their natural environment also was considered. Accurate determinations of the food intake of these fish, as well as other pertinent data, presumably will be necessary for arriving at reliable estimates of their average metabolic rates.

(Pendleton)

The cycling of cesium was discussed with the general conclusions that insufficient data are presently available concerning the cycling and distribution of cesium among the various fractions (water, colloids, silt, detritus, and biomass) in aquatic environments. At low concentrations in fresh water all cesium ions become bound to colloidal and suspended matter. The relationship of the behavior of cesium and potassium has been shown to be quite different from that of strontium and calcium. Cesium at highest trophic levels is concentrated threefold that of chemically similar potassium. In an aquatic environment, the availability of cesium adsorbed to mud and silt has not been defined. However, aquatic rooted plants have been reported to be able to break this adsorption bond. Because of the vast individual differences, maximum concentration factors for cesium should be reported for each species.

Methods were discussed for separating and measuring the percent of cesium in plankton, detritus, and the inorganic fractions. Because cesium is known to move more freely in aquatic and moist environments, questions were raised about its uptake and movement in rooted plants producing aerial shoots. Biological transport of cesium in submerged aquatic organisms is less pronounced in warmer waters of the tropics than in cooler waters, partly because of differences in the rate of diffusion that brings about a more rapid turnover in the warmer waters.

Some of the present techniques of quantitating the amount of cesium present in living cells are inadequate because they fail to measure the cesium held within membranes in cell saps. This soluble cesium has been shown to be in high concentrations within phytoplankton cells.

New waste disposal methods will have to be developed to handle the future supply of cesium-137. The present tank storage methods are not the answer. Cesium-137 is a greater genetic hazard than strontium 90 and iodine-131, which are somatic hazards. In the food web to highest trophic levels, cesium is more concentrated than most other radionuclides.

TEMPERATURE EFFECTS ON THE AQUATIC BIOTA

SUMMARY OF DISCUSSION

Extensive and intensive discussion was stimulated this paper. The most outstanding points included following:

1. Heated water below power plants attracts fish from the surrounding areas. This was corroborated elsewhere in the United States. Attracted fish included salmonoids in winter, but ran heavily in warm-water types in summer.

2. There is no information available relative to changes in productivity of fish, since the area cannot be effectively studied. Dr. Trembley feels that in the study he reported productivity was probably limited by heavy blue-green algae growths that occurred throughout the year in the heated-water area.

3. There seemed to be general agreement that life cycles are speeded up in warmer water areas. Tendipeds were reported as emerging on a year-round pattern in Pennsylvania, except for a limited period during the hottest weather. Threadfin shad were reported as spawning 1 month ahead of time in a southern river. Net-building caddis flies, Hydropsychidae, were reported to be stimulated to grow and emerge on a year-round basis below a power plant in the Muskingum River, Ohio.

4. The subject of heated waters having significant organic content (biochemical oxygen demand) was discussed. It was generally recognized that such waters might present great difficulties. A situation in Michigan was reported in which a power plant upstream from a sewage plant heated the water to a point where serious anaerobic conditions developed below the plant. This situation was ameliorated by the installation of a cooling tower and recirculation. It was the opinion of some that although speeding up natural self-purification processes in the winter might result in a beneficial reduction of sludge deposited, in summer the increased digestion rate would be more likely to result in anaerobic conditions.

5. The benefits of weirs and jetties in effecting mixing of heated waters with natural stream waters was illustrated by the situations in the Delaware River, and in the Thames River in England. Different physical principles were involved in the two cases; however, in the Delaware the hot waters were channeling along one bank, whereas in the Thames they were definitely channeling at the surface in mid-stream, eventually spreading out entirely across the stream. Information on distances that heated waters might traverse before complete assimilation into the stream in the absence of weirs or jetties was not available.

Other topics discussed included:

1. The shock effect of temperature changes on fish;
2. the desirability of considering fish management needs when planning power plant installations;
3. the reduction of dissolved oxygen at elevated temperatures;
4. the difference between the "peak load" plant, which may at "peaking" hours raise the water temperature as much as 25°F in passing through the plant, and the "base load" plant, which may raise the temperature of the cooling water only 10° to 12°;
5. an extended discussion of a power plant on the Thames River, England, in which it was concluded that "detrimental effects seem to be negligible."

Suggested needed research:

1. The normal maximum temperatures for aquatic life, including bacteria;
2. the effects of raised water temperatures on the total biota of streams and lakes in diverse areas;
3. the correlated effects of heated water, BOD, COD, and digestion of organic wastes.

EFFECTS OF POLLUTION ON OYSTERS AND FISH IN TAIWAN

Professor Pao/Shu Chang *

Taiwan is an archipelago situated in the semi-tropical zone at the crossing of the warm and cold currents. The surrounding waters flourish with rich marine resources. In recent years, however, the problem of water pollution has come very much to the fore. Rapid industrial and economic development of the province, the concentration of urban life, and the mushroom growth of plants and factories have combined to cause, in some areas, the disposal of urban refuse and industrial wastes to become serious threats to public health and aquatic life. In some harbours the dumping of all oil wastes from ships has resulted in toxic effects on aquatic organisms either directly or indirectly. It is reported that the industrial wastes in Taiwan contain large amounts of copper, sulphur, zinc, and harmful organic substances. It is also contended that the destruction of the habitat, with the elimination of plankton and bottom organisms is partly responsible for the decline in coastal fishery products in recent years.

Below are some of the scourges that are affecting Taiwan fisheries with increasing intensity.

I. Kaohsiung Bay.

Oysters flourish well on sandy flats in Kaohsiung Bay. In 1952, however, green oysters began to appear at certain locations in the Bay. When eaten they cause vomiting and diarrhea, the symptom of food poisoning. To reduce the poison, the fishermen put the oysters in pure water and washed out a certain amount of the green substance. The freshness and the firmness of the oyster, however, were lost with the washing. The oyster price dropped, but the demand dropped still further; almost to a vanishing point.

According to the report of the Taiwan Fisheries Research Institute, green oysters first appeared at Linyar Liao of Kaohsiung in 1952. The wild oyster became green first. In 1954 green oysters were found in the oyster culture beds in the vicinity of the electric power plant and fertilizer factories. By 1955, excepting the areas of Hon Mao Kung and Chi Juin where the tidal current is comparatively strong, all the oyster beds in Kaohsiung Bay were affected. The price of the green oysters dropped to only half of their original price. Statistics of the local fishermen's association show the value of annual oyster production in the above-mentioned areas at about a new total of $5.4 million. In consequence of the declining demand after water pollution, this figure fell to a new total of $2.7 million. This resulted in serious hardship to the livelihood of the coastal fishermen, brought about a cessation of oyster culture in those areas, and adversely affected all related crafts and craftsmen.

Two factors are responsible for the greenness of oysters: (1) absorption and storing of abundant microorganisms (e.g. *Navicula ostrearis*) in the gills and intestines of oysters; (2) excess of copper salt (e.g. $CuSO_4$) in the water of oyster beds. Green oysters due to factor (1) are unharmful, edible, and delicious tasting. They are also found and eaten in Europe and America. However, oysters which are green due to accumulation of copper salts are poisonous. Upon testing of specimens from the Kaohsiung Bay, it was found that the green oysters contained much green substance in their cells and tissues. However, no green microorganisms were observed in their digestive tract.

By applying the Borax ball flame test, it was found that most of the green substance was a copper salt. The copper content in the green oysters of Kaohsiung Bay was found to be 1,180 mg/kg, while copper content in ordinary oysters is only about 5.6 mg/kg. Thus the oysters from Kaohsiung Bay contain over 200 times as much copper as normal oysters.

Kaohsiung Bay is in the shape of a large bag with a narrow mouth. Numerous factories and plants have been set up along the inner shore line. Under the present conditions tremendous amounts of industrial effluents containing a considerable amount of copper salts are being dumped into the harbour. Hence, it can be confidently inferred that water pollution is the chief cause of the scourge to the oyster beds in Kaohsiung.

II. Tainan Coast.

The effects of pollution on fish along Tainan coast were first brought to notice in 1960. It was reported that the effluents from some local paper mills were causing marked damages to aquatic life in the vicinity.

Analysis of the water taken therefrom showed that the effluents contained much sodium hydroxide, paper pulp, and some strong acids. The sodium hydroxide has a direct harmful effect on fish and shellfish, and the paper pulp chokes the fish gills. Another component, hydrogen sulphide, is very poisonous to fish and harmful to their growth.

III. Shaokang Area, Kaohsiung Hsien

Shaokang is an important industrial center in southern Taiwan. The industrial effluents from the sugar factories and paper mills have become seriously detrimental to fisheries in this area.

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The analyses of the effluents have been conducted by the Taiwan Fisheries Research Institute, and the findings are tabulated as follows:

No. of sample	Location of sampling	pH value	Odor	Organic substances (Kubel Method)	Hydrogen sulphide I_2 Method	Dissolved oxygen (Winkler Method)
1	Sea water (100 m from shore)	7.2	-	0.97 g/l	4.88 mg/l	3.53 cc/l
2	Mixture of effluents and sea water	7.4	H_2S	1.14	8.17	2.40
3	Effluents from sugar factories	7.2	Methane	37.33	7.16	0
4	Effluents from paper mills	7.2	Pulp odor	2.85	5.21	3.38

The above-mentioned statement is intended to show the detrimental effects of pollution on fish and oysters in Taiwan during recent years and the growing seriousness of the problem of pollution.

The following are recommended as effective measures in dealing with water pollution: (1) strengthening of study and research on the problem of pollution; (2) insistence on the proper design for effluent equipment of all plants and factories before approval is granted; (3) strengthening of effluent control of factories already established; and (4) strict prohibition of the dumping of oil and other wastes from ships into harbour and inland waters.

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SUMMARY OF THIRD SEMINAR

Richard H. Stroud and Charles H. Callison

In an attempt to give our summarization direction, we asked ourselves: What is the purpose, or function, of a seminar in a given area of scientific endeavor? The purpose is multiple because several answers can be given. The seminar is a device for becoming up-to-date: for discovering what is being done and what has been found out by other workers in other institutions, in other lands, and in related fields of study. It is an opportunity for the exchange of technical data and of information about new techniques and equipment. It is a place for mutually helpful questioning and criticism: the mass application, so to speak, of the principle that two or more heads are better than one.

The seminar also offers a medium for the identification of trends, new needs, or new directions for investigation and for the discernment of blind alleys from which research must turn in its never-ending quest for the roads to truth. Much must continue to be said for the essential value of basic research; but, at the same time the total research effort has an inevitably pragmatic cast, particularly when it is dictated by a massive social and economic need such as the world wide problem of water pollution.

It was appropriate that this seminar devote some attention to what might be called the "environment of research." We refer to the social, economic, and political factors that affect your jobs, your appropriations, and your opportunities to work.

Finally, the seminar offers an opportunity for generalization, and in this summarization we are attempting in a way to be generalists. We have tried to express some general conclusions, all of which you may not agree with but which may stimulate you to do some general thinking of your own in a search for more valid conclusions.

Most of you are specialists in a rather narrow field of science. It is said that as scientists become more specialized they lose the ability to communicate successfully with specialists in other fields. And what may prove a more serious handicap, they lose the ability to communicate with the public and with the politicians and administrators, in both industry and government, who by the very nature of their jobs are required to be pragmatists and to possess, or develop to a high degree, the mental outlook and skills of the generalist.

The public, the politicians, and the administrators are the ones who create the "environment of research." They use, or fail to use, the findings of the research specialist. They provide the money and the facilities for your training and your work. These facts were recognized by the organizers of the seminar program. The opening speech was delivered by James M. Quigley, Assistant Secretary, U. S. Department of Health, Education, and Welfare, whose career has illustrated the importance of the generalist in our society. He has been a successful politician, a member of Congress, and now his work includes the administration of the federal responsibilities in water pollution control.

The Monday morning panel was composed of administrators and generalists, and their subject was how the findings of research are being used, or can be used, in keeping waters fit for human uses.

We shall undertake to point out some of the significant thoughts and problems expressed in the panel on water quality criteria. Next, we shall try to summarize the other panels and record some impressions of the concurrent, informal dicussions. We regret that it was physically impossible for two people to sit in on all of them. And, we beg of you, please do not infer any slight or intentional oversight if we fail to mention your name or your paper. The time available for this summarization and the physical format of the seminar made it impossible to include all of the splendid papers that truly deserve recognition. Some omissions are due simply to the fact that not all of you submitted copies or digests in advance.

This summarization is the best effort, in a limited time, of only two people. When the transactions are published, we urge each of you to read them in full and form your own conclusions. A far better job than this one--and a more valuable job to each of you personally--can be done at your leisure.

Finally, we shall undertake some general conclusions and observations. The emphasis of Secretary Quigley's speech should have special and encouraging significance to this particular group of professionals. The problem of water pollution, he said, is broader than its public health aspects. This may not sound new to you, but its utterance is significant in view of some jousting now going on in Washington and some other places.

The uses man makes of water are mostly biological and, therefore, the concern of biologists. Your approach, he recommended, should be ecological, and you can help by continually stressing that it is not the average but the extremes of conditions that are important and are the true measures of the suitability of an environment.

He pointed out that on the basis of new legislative authorizations for research and enforcement that have not yet come to fruition the politicians in Congress have been ahead of the doctors, lawyers, engineers, and scientists in the recognition of the massive effort that must be mounted to overcome pollution. This must mean the public is taking the lead, and if this is so, it is a most healthy situation.

Finally, the Secretary signed up the biologist as a regular member of the team--something none of you here has had any doubts about although others may be inclined to relegate you to the minor leagues --but he warned it is up to the biologist to decide whether he becomes a star or proves merely to be a bench warmer.

Opening the panel on water quality criteria, Mr. Murray Stein, whose reputation for vigor and skill in federal enforcement actions has been justly earned, expressed the opinion that criteria, like time and the tides, are inevitable. In later discussion, however, he seemed to reveal some personal doubts about them.

"We should guard," he said, "against the development of criteria becoming the main goal of our professional life." Your summarizers prefer to interpret this statement as meaning that Mr. Stein believes that, pending the development of scientific criteria, the administrators and policymakers should not use the absence of such criteria as an alibi for postponing action on flagrant cases of pollution that should be cleaned up now through enforcement action. Dr. Tarzwell, of course, emphasized at several points along the way that criteria should be developed on the best available basis and refined later.

Michigan's "old pro" administrator, Mr. Milton P. Adams, sees water quality criteria most useful as administrative aids and in comprehensive planning as authorized by Section 2 (a) of the Federal Act, but of doubtful usefulness in enforcement. The administrator, he said, needs criteria not just for the protection of aquatic life but for all lawful uses of water.

Mr. Weston's contribution was a forthright and understandable exposition of the point of view of the industrialist. Criteria are absolutely necessary, from this point of view, in order that industry may know what is expected of it. He did not discount the possibility that corporation executives may challenge and dispute such criteria as are finally developed by public agencies, and he explained why it must be the policy of management to keep expenditures for waste treatment at the minimum and to postpone such expenditures as long as possible.

Mr. Kimball presented an equally forthright statement of the viewpoint of the conservationist, who calls for the speedy development of scientific criteria as essential to an effective attack on pollution. There is no water problem too difficult, he believes, if research is given adequate manpower, money, and the other tools to tackle it.

The discussion that followed, including some excellent contributions from the floor, was sharp, interesting, and divulged wider cleavages in philosophy than may have been apparent in the prepared statements of the panelists.

Now turning to the other panels: At the present time, the environmental requirements of most aquatic organisms, for protozoa and algae to fish, in both fresh and salt water, are poorly known at best. Only the broad parameters of the more obvious environmental factors such as dissolved oxygen content, pH, temperature, salinity, and a few others have been assesed for rather restricted numbers of species, generally as single variables rather than as multivariates. For the most part, emphasis has been placed on lethality over short periods of time in terms of averages. Yet, the consensus of the panelists seemed to be that an ecological approach, emphasizing multivariate relationships, is needed. It was recognized that an animal, or species, or community, can as surely be destroyed by gradual change of the environment in subtle ways as by sudden development of directly lethal conditions.

The use of averages is important for general understanding of quantitative data, of course, but the average of environmental conditions is not a measure of the suitability of an environment. Rather, as Mr. Quigley pointed out, it is the extremes that are important and yield the true measures. Much work needs to be done in this area.

In this respect, the paper by Mr. Alderdice on multivariate analysis of experimental environments was particularly pertinent. Only in this way can precise definitions of pollution be developed that will be meaningful in pollution abatement work, whether it be treatment of wastes or enforcement of laws. Mr. Adams had complained on Monday of the frequent inability to make antipollution laws work because of a lack of precise definitions and pointed to the biologists as the ones best equipped to secure the needed answers. Mr. Alderdice holds--we believe correctly--that such answers are relative. With reference to such questions as: "How much oxygen does a fish need?" or "What is the highest concentration of a particular pollutant that may be tolerated?", he states that the answers are "relative to the other factors and their levels that may be impressed simultaneously on the particular animal considered." Various combinations of levels of the factors serve to impose lesser or greater stress in the presence of a pollutant. Better comprehension of the functional capacities of organisms to meet environmental changes should result from the multivariate approach.

Microorganisms capable of converting, into useful products, the waste organic materials making up the residue of so-called complete treatment processes are badly needed to save part of the vast quantities of dilution waters now required. Mr. Kimball pointed to the profligacy of precious water inherent in our present lack of knowledge along these lines. Dr. Hutner, in a remarkably lucid and provocative lecture, cited the need for microbial degradation of many pollutants and suggested an approach through research on photosynthetic bacteria.

Much emphasis was placed on the utilization of various forms of plant and animal life as "indicator species." This method has been used with considerable success to suggest the need, or lack of need, for intensive studies. Various specialists pointed out both advantages and limitations of this approach. At the same time, much emphasis was placed on the need for specific information on water quality requirements. For example, the insect panelists were unanimous on this point, being able to cite few exceptions and then only for the more perceptible factors.

Dr. Fjerdingstad's paper on a new saprobic system (particular reference to algae) was especially cogent, suggesting more attention be paid to community aspects of organisms. He suggested that community formation must be analogous to optimal living conditions of the species and that this may be more sensitive than the conventional use of indicator species, the individuals of which have wide tolerances. The presence of vitamins and other organic compounds must be accounted for as well as temperature, nutrient salts, etc. Various metabolites that may be released from algal cells exert a variety of biological effects upon other organisms. Dr. Fogg discussed the role of glycolic acid as a necessary factor for the growth of *Chlorella* and other microorganisms. Dr. Gorham showed that the development of a toxic waterbloom depends upon the favorable interaction of at least six variables.

A review of the literature on environmental requirements of shrimp by Dr. Broad established that the requirements are poorly known, that there is an acute need for physiological research, and that many insecticides are highly toxic to shrimp. Dr. Butler's work of more than a decade on a population of commercially unexploited oysters in a northwest Florida estuary revealed that apparently subtle and largely unidentified environmental factors may drastically alter the character and quality of oysters. Significant multivariate studies by Dr. John D. Costlow, Jr., of the influence of three or four enviornmental factors on crab development also suggest both the complexities and the urgency for delineating standards of water quality for marine organisms. This work points to the possible use of crab larvae as bioassay animals for studies of marine water quality and pollution.

Mr. Pentelow noted that the characteristics of the aquatic environment undergo gradual natural change, but that man affects the nature of such changes. We must live with modern developments and do the research that will permit evaluation and abatement of the effects of those developments. He cited the discharge of waste heat into rivers and of substances directly toxic to fish and other organisms. Drs. Doudoroff and Warren described effects upon fish embryology, growth, and certain aspects of physiology, of different dissolved oxygen concentrations, temperatures, and water velocities. They also discussed the need for research into the ecological significance of these requirements of fishes and some of the problems involved in these and other water quality requirements, most of which are poorly known at best. Dr. Bennett confirmed this for centrarchid basses. Dr. Huet stated that relatively clear-cut criteria can be expressed for temperature, dissolved oxygen, and pH for only a few species of fish. He said, "The results of many past experiments (about the toxicity of various substances in complex water) are of little use because the composition of the water with which they were carried out is not known."

The modern agricultural, industrial, and recreational pollutants inject new dimensions into the water quality picture. Unfortunately, little is known quantitatively about either their acute or chronic effects. The toxicity to fishes of such substances as synthetic detergents (Vivier and Nisbet), outboard motor exhaust wastes (Surber, English, and McDermott), oil pollution (Erickson), heavy metal salts (Lloyd), salinity (Kinne), and organic insecticides (Nicholson) is further evidence of the need for polyfactorial analyses in evaluation of water quality criteria. Mr. Lloyd stated the need well: "The ultimate aim of laboratory bioassays is to enable one to predict a concentration of poison or effluent that must not be exceeded in a river if fisheries are to remain unharmed... the measure of toxicity used has been the lethal threshold concentration, since this value can be accurately determined, but more information is required of sublethal effects that may result in a gradual deterioration of the condition of the fish. . . . It is only by a more complete understanding of the basic mechanisms involved in the relation between the susceptibility of fish to poisons and to changes in the environment that any real advance can be made."

Since World War II a whole new spectrum of pollutants--the radionuclides--has appeared in surface waters as a result of the production and use of fissionable materials. A number of these elements are naturally present in aquatic environments but are now increasing. Discussions here revealed a general paucity of information concerning this new field of inquiry. Limited observations by Dr. Ravera on normal radionuclide content of freshwater organisms and sediment in Lake Maggiore in northern Italy revealed widely different values of manganese -54 in mussels in different zones. Uptake and retention of the radioactive contaminants by marine plankton could be postulated only on theoretical grounds, according to Dr. Lowman. Mr. Price reported a few mollusks may accumulate at least some radioisotopes in all parts of the body and exoskeleton in amounts that vary with both the nuclide and the species involved. Several years work on Hanford reactor effluent and receiving waters of the Columbia River have resulted in an outline of some parameters of the relationships between radionuclides and some species of invertebrates and of fish, as reported by Dr. Davis and by Dr. Foster and Mr. McConnon, respectively, but this area of inquiry is in its infancy. In such work, a large number of samples taken over at least a full year is needed, because of great variability in uptake of radionuclides by fishes, to yield an estimate of the average radionuclide concentration in fishes and a picture of the fluctuations with time. For muscle tissue of Rocky Mountain whitefish, seasonal variations in the concentration of phosphorus -32 are on the order of 50-fold, and that of zinc -65, 10-fold. Concentration factors in the whitefish ranged from zero to 5,000 times those found in water for phosphorus-32 and up to 1,000 times for zinc -65. Both phosphorus -32 and zinc

-65 are present in relatively low concentrations in the river but, being biologically important, are dominant in the fish. In contrast, the really abundant nuclides in Columbia River water (Chromium -51, arsenic -76, and neptunium -239) are relatively unimportant in fish.

Again, we wish to express our regret that it is neither possible nor appropriate to discuss all the fine papers that were presented in the stimulating concurrent sessions. Among them, however, we noted with particular interest that Dr. H. B. N. Hynes advanced the thesis that low levels of pollution can be rapidly detected by determining the numerical makeup of the macroinvertebrate population in a stream. For example, a gross initial survey that shows a decline in numbers of plecopterans and an increase in ephemeropterans in a British stream as one moves downstream indicates a pollution source near by and suggests the extent and magnitude of its effects. By quantitative serial sampling, done on the same type of substrate for comparison, Dr. Hynes noted that relative numbers of the two invertebrates gave clear evidence of biological alteration even where pollution was slight.

Dr. Cope pointed out that methods of analyses of many herbicides by chemical techniques have not yet been devised. Present bioassay methods determine toxicity only semiquantitatively, by comparing standard growth or mortality curves. The accurate measurement of pesticide quantity appears to be vital to the proper determination of toxicity patterns. Many difficulties are encountered in laboratory analyses. Highly variable results are obtained when individual animals are tested. To understand and predict the effects of a pesticide on fish, one must know how its action is affected by water hardness, alkalinity, pH, and other factors; what its effect is on fish-food organisms; what the differences in chronic and acute toxicity are; and other factors.

The problems posed by pesticides are many and complicated, and the research that needs to be done is of staggering proportions. For example, Mr. Bridges reported that in measuring the toxicity of heptachlor and keptone to redear sunfish *(Lepomis microlophus)* changes in resistance of the same lot of fish occurs during the holding period, and different lots of the same species of the same size exhibit great differences in resistance under identical conditions. Ranges in chemical concentrations to produce different effects are very narrow.

Fortunately, some new techniques are being developed to measure insecticides in water samples. Mr. Van Valin and Dr. Kallman described the use of small quantities of carbon (charcoal) for absorbing pesticides from water. In practice, 1-gallon samples of water are shaken with 10 grams of charcoal. The charcoal is filtered off, briefly air-dried, extracted, and analyzed by paper chromatography. Recoveries of 60 to 90 percent of heptachlor have been achieved in the 25- to 500-microgram range.

Dr. Hoffman stated that pesticide pollutants can be controlled by federal and state laws specifying conditions of use, by supervision of pest-control programs by trained and responsible operators, by establishment of pest-control review boards, and by educational activities and "good public relations" in connection with the general safe use of agricultural chemicals. He emphasized the need for research to discover less toxic pest control compounds or, more desirably, nonchemical methods that would obviate water pollution problems.

Dr. Nicholson reported that toxaphene and the gamma isomer of benzene hexachloride (BHS) were detected nearly year-round in waters draining from a 400-square-mile river basin where cotton is the main crop and occupies only about 6 percent of the land in the watershed in any given year. He stated that occurrence of sublethal quantities of pesticides in surface waters may be rather common in areas of routine pesticide usage. The concentration at which harm is caused by such pollutants remains to be learned since evaluation of chronic-exposure effects at low levels is very difficult. Much more work in this area is critically needed.

The need for more exact and sensitive procedures was stressed also by Mr. Burdick. He cited the needs for a system of screening chemical compounds, improved information on synergy and antagonism procedures for post mortem analysis, and mathematical models permitting evaluation and correction for simultaneous interaction between components of the toxic system. The problems in fish appear to be much more complex than in man.

Dr. Patrick observed that the general use of pollution-indicator species has become much more difficult because of the rapidly increasing number of polluting substances that have very different chemical and physical characteristics. Furthermore, variations in the natural environment may greatly influence population size of "indicator species" or even their presence or absence. The importance of considering the structure of the whole algae community *as well as the kinds* composing it has been emphasized in recent years.

Dr. Snieszko and Dr. Glenn L. Hoffman propounded the need for test fish of uniform quality. Parasitic, bacterial and viral diseases, malnutrition, and uncertain hereditary background, singly or in combination, can drastically affect the usefulness of the fish for bioassays. They outlined fish-disease control by drugs, breeding of disease-resistant strains, and general sanitary measures. They noted the inadequacy of these measures and proposed the establishment of "fish-breeding institutions" to provide uniform, disease-free fish such as are now available to supply some laboratory animals; e.g., white mice, rats, and guinea pigs.

Long-term studies of the physiological responses of aquatic animals to pollutants were urged in a paper by Dr. Fromm and in the discussion session by some of his colleagues at Michigan State University. Dr. Fromm described an assessment procedure, involving the measurement of oxygen consumption of assay animals, that is based on the assumption that no matter when environmental stress is placed on an animal it will be reflected in a change in normal metabolic rates.

Dr. Isaac recounted the original development of the BOD technique by the Royal Commission on Sewage Disposal (1898-1915) and the complicating factors introduced by high suspended solids and bottom muds. The Royal Commission proposed the standard of 20 ppm BOD and 30 ppm suspended solids as maximum for treatment effluents, which has been almost universally applied in England although without statutory support. The BOD standard has fostered much research on the fundamentals of aerobic stabilization of organic matter, but relatively little attention has been given to the suspended solids standard. Suspended solids and bottom muds interfere with the BOD test and represent important gaps in our knowledge.

Your summarizers moved about among the concurrent sessions in a kind of sampling process; and, in general, we found the discussions interesting, the discussers keen, and the give-and-take free and frequently blunt. We can understand why such sessions have become a fixed feature of this seminar.

Among the recurring emphases and concerns expressed throughout the conference, we see two definite and healthy trends. The first is a turning toward an ecological approach in the quest for new and practicable parameters in the detection and evaluation of pollution and for ways of combating it. The second, a corollary of the first, is a movement toward greater dependence on field studies.

There was a time when the classical biologists, the men who controlled the academic institutions and standards, expressed unveiled contempt for "field research," brushing its findings aside as vague, inexact, and impossible of controls, and in the same gesture brushing aside those who would study nature "in the raw" as dreamers, as indolent piddlers, or, the ultimate dismissal, as mere "naturalists."

But all too frequently the "laws" or "rules" or calculations arrived at in the laboratory failed mysteriously to work in the rivers, as was pointed out here by Dr. Kinne and others. Messrs. Foster and McConnon made a particular point of the need for field studies and for experiments that "closely simulate field conditions," because there are so many variables, and so many possible combinations of variables, in nature.

The turning toward the ecological approach was reiterated by Dr. Cairns, by Dr. Broad, by Dr. Erickson, by Dr. T. T. Macan, and by others whose papers have already been mentioned. The questions asked by Dr. Alderdice and quoted earlier in this summary must also be asked in other ways, as what concentration of a pollutant may be tolerated *for how long?* and with reference not to a particular animal or plant but to a *community of living organisms.*

These apparently are the things about the life sciences that baffle and prompt the impatience of the chemists and engineers, whose disciplines are based almost entirely on conditions subject to precise control and measurement in a laboratory. But if there is a breakdown of communications caused by a misunderstanding of basic concepts between the chemists and the biologists, there is a suggestion of the same trouble between specializations in the life sciences. We trust it is only *suggestive* of such trouble that we had here one paper arguing for the use of the larger invertebrates as indicators of pollution, others pursuing the importance of the diatoms and other algae, and still others stressing the aquatic insects or some other group of organisms. To the practical research director or program administrator, it must be apparent that each of these methods is potentially valuable and each must be developed not as the only key but as one of a kit of tools that may be used, singly sometimes but more often in combination, in the ultimate determination of practical criteria of water quality. This was surely in Dr. Fjerdingstad's mind as he undertook to construct a "new saprobic system."

Then we heard in this seminar a call, almost plaintive, for research in depth as to time as well as to ecological relationships. The papers by Dr. Fromm and Dr. Wilber, among others, suggest the need for advancement, at least in the American research world, beyond the system of ad hoc research and research by short-term grant.

What is wrong with the system that finds us only now beginning the most rudimentary research into the ecological and long-term side effects of the synthetic organic pesticides full 2 decades after the invention of DDT, after the chemical pesticides industry has reached an annual sales volume of $300 million in the United States alone? Who has failed to meet his responsibility? We are still trying, on meager budgets and with a mere handful of government personnel, to discover methods of detecting the presence of these chemicals in water while concurrently, we may assume, industry is spending millions in the development of new pesticides and other millions in their promotion and sales.

"In addition to money," said Dr. Fromm in his pleading for long-term physiological studies in determining sublethal effects, "two things are needed for research of this type to be carried out: (1) the favorable attitude of administrators and (2) trained personnel to do the work."

This brings us to some concluding observations on the subject of communications. Communications is perhaps more of an art than a science but any scientist with average intelligence and a tongue in his head can master the fundamentals of it. We speak of the need for research biologists to learn how to get their story across--to each other, to their bosses, to the legislators and boards of corporations, and, most important of all in the long run, to the public.

It is never too late in life, for example, to improve one's speaking ability. The manner of the presentation of papers here ranged from very good to very poor.

One does himself and his profession an injustice when, in making a speech or reading a paper, he ducks his head and mumbles inaudibly into his shirt front.

Not every biologist can be a gifted writer like Rachel Carson, but any one of you can take the time and trouble to make friends with the public relations specialist in your agency or company and to help him get your story to the press. Any biologist can arrange to take administrators or civic leaders or newspaper and magazine writers on a tour of the laboratory or field project, letting them see with their own eyes the importance of your work. These are just a few of the methods that will work.

We shall venture the opinion that the current article in Readers Digest about Dr. Ruth Patrick will do more to assure continued public and financial support for the work of the Philadelphia Academy of Natural Sciences than all the papers presented at this seminar by members of the Academy staff. Please never, through false modesty, or genuine modesty, or sheer inertia, or for other reasons, pass up an opportunity to get similar publicity, even in your home town newspaper. And don't overlook the wisdom of cutting your chief or your administrator in on the publicity.

It was a pleasure and a privilege to serve as your summarizers. We hope all of you found the seminar equally profitable. The attendance itself, which we understand from Dr. Tarzwell was a record of 436 registrants, including participants from 26 foreign countries, augurs well for the future of aquatropic man.

ABSTRACTS

ENGLISH

THE VALUE AND USE OF WATER QUALITY CRITERIA TO PROTECT AQUATIC LIFE IN STATE PROGRAMS. Milton P. Adams. p. 7 . The value and use of water quality criteria for the protection of aquatic life are discussed from the point-of-view of a state administrator. The question is raised as to whether the use of water quality criteria should be limited to the protection of aquatic life. If the state administrator needs criteria for the protection of aquatic life, criteria are needed equally for the protection of other lawful uses of the water of his state.

Most state administrators necessarily regard criteria or objectives or standards as means of facilitating compliance with the state laws. State laws with their varied prohibitions or lack of them do not contain such things as water quality criteria. Perhaps this is because legislators do not understand such things as DO, BOD, and MPN, or because of the widely varying concepts of what water qualities are injurious to public health. In many cases, water quality criteria set up as compliance aids have failed for one reason or another to provide results. Water quality criteria can best be used as administrative rather than as enforcement aids, and to implement provisions of the law.

The interrelationship between federal, interstate, state, municipal, and industrial efforts to obtain certain results through comprehensive programs for water pollution control clearly require soundly conceived criteria that are susceptible of fulfillment. Lack of knowledge impedes the development and use of water quality criteria for protecting aquatic life. Basic and applied research is needed to supply the enforcing agencies with solid answers to back up their position when restricting waste loadings.

FRENCH

LA VALEUR ET L'UTILITÉ DES CRITÈRES DE QUALITÉ D'EAU POUR PROTEGER LA VIE AQUATIQUE EN PROGRAMMES D'UN ETAT. Milton P. Adams. p. 7 La valeur et l'utilité des critères de qualité de l'eau pour protéger la vie aquatique sont discutées du point-de-vue d'un administrateur d'état. La question est posée si l'emploi des critères de qualité de l'eau pourrait être limité à la protection de la vie aquatique. Si l'administrateur d'un état a besoin de ces critères pour la protection de la vie aquatique, des critères sont également requis pour la protection des autres emplois légitimes de l'eau de son état.

La plupart des administrateurs d'état considèrent nécessairement les critères, ou objectifs, ou les modèles comme un moyen de facilité conformément aux lois des états. Les lois des états avec leurs interdictions varient ou leur manque de celles-ci ne contiennent pas de tel sujet comme celui des critères de qualités de l'eau. Peut-être c'est parce que les législateurs ne comprennent pas de tels choses comme DO, BOD, et MPN ou parce que vu la conception diverse de quelques critères de qualité d'eau ce serient nuisible à la santé publique. En beaucoup de cas, les installations pour la recherche des critères de qualité de l'eau ont failli pour une raison ou pour une autre fournir des résultats. Les critères de qualité d'eau pauvent mieux être employés sur le plan administratif au lieu de l'employement des aides pour l'exécution des dispositions de loi.

Les relations entre la Fédéral, l'entre-état, l'état, les municipalités et l'industrie pour obtenir certains résultats à travers des programmes comprehensifs pour la contrôle de la pollution d'eau demande précisement des critères solidement concu qui sont susceptibles d'aboutir. Le monque de connaissance retarde le développement et l'emploie des critères de qualité de l'eau pour protéger la vie aquatique. Les recherches élémentaires et appliquées fournissent l'agence d'exécution avec des responses solides afin d'appuyer leur position au sujet des restrictions de depôt d'ordure.

GERMAN

WERT UND ANWENDUNG VON WASSERGÜTEKENNZEICHEN ZUM SCHUTZ DES LEBENS IN WASSER IN STAATLICHEN PROGRAMMEN. Milton P. Adams p. 7 Wert und Anwendung von Wassergütekennzeichen zum Schutz des Lebens im Wasser werden vom Standpunkt des staatlichen Verwaltungsbeamten besprochen. Die Frage wird aufgeworfen, ob die Anwendung solcher Kennzeichen auf den Schutz des Lebens im Wasser beschränkt bleiben soll. Wenn der staatliche Verwaltungsbeamte diese Kennzeichen für den angegebenen Zweck nötig hat, dann braucht er gleicherweise Kennzeichen für den Schutz anderer gesetzlicher Anwendungen von Wasser in seinem Staate.

Die meisten Verwaltungsbeamten betrachten notwendigerweise Kennzeichen, Ziele oder Normen als Mittel zur Erleichterung der Anwendung der Staatsgesetze. Staatsgesetze mit ihren verschiedenen Verboten oder der Abwesenheit von Verboten enthalten solche Dinge wie Wassergütekennzeichen nicht. Vielleicht ist das darauf zurückzuführen, dass Gesetzgeber Abkürzungen wie DO, BOD oder MPN nicht verstehen oder weil die mannigfaltigen Ideen über Wassergüte der öffentlichen Gesundheitspflege schaden könnten. In vielen Fällen schlugen Wassergütekennzeichen, die zur Erleichterung der Gesetzhandhabung eingeführt worden waren, aus nicht näher bekannten Gründen fehl. Solche Kennzeichen können am besten als Behelfe der Verwaltung und als zusätzliche gesetzliche Verordnungen benützt werden, nicht aber für polizeiliche Massnahmen. Die Beziehungen zwischen Bundes-, zwischenstaatlichen, staatlichen, gemeindlichen und industriellen Bemühungen zur Erlangung bestimmter Ergebnisse durch umfassende Programme zur Kontrolle der Wasserverunreinigung verlangen offensichtlich klar durchdachte und praktisch erreichbare Normen. Mangelndes Wissen hindert die Entwicklung und Anwendung von Wassergütekennzeichen zum Schutz des Lebens in Wasser. Grundlagen-und Zweckforschung sind nötig, um den Vollzugsorganen zuverlässige Angaben an die Hand zu geben, auf Grund deren sie Massnahmen zur Einschränkung der Belastung von Gewässern durch Abflüsse ergreifen können.

ANALYSIS OF EXPERIMENTAL MULTIVARIABLE ENVIRONMENTS RELATED TO THE PROBLEM OF AQUATIC POLLUTION. D. F. Alderdice. p. 320. By means of a modified scheme of factorial analysis, the response of terms of median resistance time in minutes of juvenile coho salmon to 3 milligrams per liter of sodium pentachlorophenate was investigated over combinations of levels of salinity (^{o}/ooS), temperature (^{o}C), and dissolved oxygen (mgO_2/l),

The data are fitted to a second-order polynomial that is reduced to its equivalent geometric form for evaluation of response surfaces in the three-factor system. Hence, the response to the pentachlorophenate is

$$Y_p = 111.1478 + 6.9025\, x_1 - 7.0395\, x_2 + 2.4953\, x_3 - 3.8914\, x_1^2 - 14.5269\, x_2^2 - 7.3060\, x_3^2 + 0.4918\, x_1 x_2 + 0.1298\, x_1 x_3 - 2.0013\, x_2 x_3$$

where x_1, x_2, and x_3 refer to the coded variables salinity, temperature and dissolved oxygen, respectively, and geometric form.

$$Y_p - 110.4030 = - 3.8852\, X_1^2 - 14.6689\, X_2^2 - 7.1702\, X_3^2$$

X_1, X_2, and X_3 are functions of the coded variables.

A region of optimum resistance time is computed with its center at 9.50^{o}/ooS, 3.89°C, and 5.92 mgO_2/l. Loci on the ellipsoidal envelope surrounding the optimum, at which 90 percent of the optimum response would be retained, are computed in terms of combinations of levels of salinity, temperature, and dissolved oxygen.

Functional capacities as measured experimentally are discussed with reference to their significance in the normal environment. The data indicate that within normally viable limits of the three factors tested there are combinations of levels of the factors that impose lesser or greater stress in the presence of a pollutant. The significance of this fact is discussed with reference to the problems of definition of the biological effects of water quality alteration, and the interpretation of such changes in the polluted environment.

ANALYSE DES MILIEUX EXPERIMENTAUX À PLUSIEURS VARIABLES PAR RAPPORT AU PROBLÈME DE LA POLLUTION DES EAUX. D.F. Alderdice. p. 320. En utilisant une modification de l'analyse factorielle, on a étudié les réactions des jeunes saumons coho, exprimées comme durée de résistance médiane (en minutes), à 3 milligrammes per litre de pentachlorophénate de sodium. On a étudié ces réactions per rapport à des combinaisons de conditions de salinité (^{o}/oo S), de température (°C), et d'oxygène dissous (mg O_2/l).

Un polynôme du second degré a été appliqué aux données et a été réduit à une forme géométrique équivalente pour l'évaluation des surfaces de réactions dans un système à trois dimensions. Ainsi la réaction au pentachlorophénate est

$$Y_p = 111.1478 + 6.9025\, x_1 - 7.0395\, x_2 + 2.4953\, x_3 - 3.8914\, x_1^2 - 14.5269\, x_2^2 - 7.3060\, x_3^2 + 0.4918\, x_1 x_2 + 0.1298\, x_1 x_3 - 2.0013\, x_2 x_3$$

ou x_1, x_2, et x_3 représentent les variables (exprimées en code) salinité, température, et oxygène dissous respectivement, dont la forme géométrique est

$$Yp - 110.4030 = - 3.8852 X_1^2 - 14.6689 X_2^2 - 7.1702 X_3^2$$

où X_1, X_2, et X_3 sont functions des variables x_1, x_2, et x_3.

La région de la durée maximum de résistance a été calculée comme ayant son centre à 9.50 ^{o}/oo S, 3.89°C, et 5.92 mg O_2/l. On a ainse calculé sur l'enveloppe ellipsoïdale autour du point maximum les lieux géométriques de 90% de la réaction maximum. Ces lieux ont été exprimés comme combinaisons de niveaux de salinité, de température, et d'oxygène dissous.

On discute les capacites fonctionnelles des poissons mesurées expérimentalement et leur signification dans un milieu normal. Les données indiquent ceci: en dedans des limites, normalement non fatales, de chacun des trois facteure étudiés, il y a des combinaisons de concentrations de ces facteurs qui causent des tensions plus ou moins fortes en présence d'un agent polluant. On discute de la portée de ce fait sur une définition expérimentale des effects bioligiques de l'altération des propriétés de l'eau, et de l'interprétation de tels changements dans un milieu pollué.

ANALYSE VON MEHRFACH VERÄNDERLICHEN VERSUCHSFELDERN UND DAS PROBLEM DER WASSERVERUNREINIGUNG. Don F. Alderdice. p. 320 .
Durch ein abgeändertes Schema der Näherungsanalyse wurde die Reaktion von Cohe Salmsetzlingen (ausgedrückt in mittlerer Widerstandszeit in Minuten) gegenuber 3 milligram per Liter Natriumpentachlorphenat in Verbindung mit Salzgenhaltstufen (0/oo S), Temperatur (°C.) und gelöstem Sauerstoff (Milligram per Liter) studiert.

Durch geeignete mathematische Behandlung der Ergebnisse erhält man für die Reaktion auf die Anwendung von Pentachlorphenat Y_p = 111,1478 + 6,9025x_1 - 7,0395x_2 + 2,4953x_3 - 3,8914x_1^2 - 14,5269x_2^2 - 7,3060x_3^2 + 0,4918x_1x_2 + 0,1298x_1x_3 - 2,0013x_2x_3, worin x_1, x_2, x_3 die Veränderlichen Salzgehalt, Temperatur und gelösten Sauerstoff bedeuten und die geometrische Form Y_p - 110,4030 = - 3,8852 X_1^2 - 14,6689 X_2^2 - 7,1702 X_3^2. X_1, X_2, X_3 sind Funktionen der Veränderlichen.

Ein Gebiet bester Widerstandszeit wird errechnet mit dem Mittelpunkt bei 9,50‰ S, 3,89° C. und 5,92 mg O_2/l. Örter auf der ellipsoiden Einhüllenden um den Bestwert, wo noch 90% des Bestwerts herrschen, werden für Stufen des Salzgehalts, die Temperatur und den gelösten Sauerstoff ausgewertet.

Experimentell gemessene Werte von Fähigkeiten wreden unter Bezugnahme auf ihre Bedeutung in der normalen Umgebung besprochen. Die Ergebnisse zeigen, dass es innerhalb der Grenzen der drei geprüften Faktoren Kombinationen von Stufen der Faktoren gibt, welche in Gegenwart einer Verunreinigung dem System einen mehr oder minder grossen Zwang auferlegen. Die Bedeutung dieser Tatsache wird im Zusammenhang mit den Problemen der Definition biologischer Wirkungen auf Veränderung der Wassergüte und der Erklärung derartiger Veränderungen in einer verunreinigten Umgebung besprochen.

AN EXPERIMENTAL ANALYSIS OF THE FACTORS RESPONSIBLE FOR THE PERIODIC MORTALITY OF *TILAPIA MOSSAMBICA* PETERS IN BUSHVELD RESERVOIRS IN THE TRANSVAAL. B. R. Allanson, M. Ernst, and R. G. Noble. p. 293. The cause of periodic mortality among fish in the dams of the bushveld - highveld transition region in the Transvaal, South Africa, has been investigated experimentally in the laboratories of the National Institute for Water Research and the Department of Nature Conservation, Transvaal Provincial Administration.

Many of these dams receive effluent from sewage works and industries via the inflowing rivers. Both these sources have been blamed for the periodic mortality, especially among individuals of *T. mossambica*. But the fact that such mortalities were more frequent during the cold dry winters suggests that decrease in water temperature during the winter might play a more important role than the effluent discharge into the catchments of the dams. To this end both the high temperature and low temperature tolerance and resistance of *T. mossambica* have been investigated.

Studies have shown that *T. mossambica* is capable of very rapid acclimation to high temperatures and that the ultimate upper lethal limit is slightly above 38°C. On the other hand acclimation to low temperature is very slow and as yet no ultimate lower lethal temperature has been measured. This slow acclimation to low temperatures is also linked with a high sensitivity to such temperatures (between 8°-15°C), depending upon the total dissolved solid concentration or osmotic pressure of the water during the exposure. LD_{50} estimates based upon temperatures that initiate secondary chill coma indicate clearly that the water temperature obtaining in these dams during winter is the factor responsible for the extensive mortalities, especially among the small size group (12 cm) of this cichlid species.

ANALYSE EXPÉRIMENTALE DES FACTEURS RESPONSABLES DES MORTALITÉS PÉRIODIQUES DE *TILAPIA MOSSAMBICA* PETERS DANS LES RÉSERVOIRS DES TERRES BASSES AU TRANSVAAL. B.R. Allanson, M. Ernst, et R.G. Noble. p. 293 . Les services administratifs provinciaux du Transvaal ont organisé des travaux afin de déterminer la cause de la mortalité apparaissant périodiquement chex les poissons des barrages de la région de transition entre les terres basses et les plateaux élevés au Transvaal, Afrique du Sud. Ces travaux de recherches ont été entrepris par les laboratoires de l'Institut National des Recherches aquatiques et ceux du ministère de la Conservation naturelle.

Des eaux d'origine industrielle et des eaux d'égouts se déversent dans plusieurs des rivières qui alimentent ces barrages. On a blâmé ces deux sources de pollution pour la mortalité qui semble se produire périodiquement surtout chex les individus de l'espèce *T. mossambica*. Toutefois, ces mortalités se produisent plus souvent durant les hivers froids et secs, et ceci semble suggérer que la basse température de l'eau durant l'hiver jouerait un rôle plus important qui le débit des eaux polluées dans les bassins de drainage en amont des barrages. C'est pourquoi on a entrepris des travaux sur la tolérance et la résistance de *T. mossambica* aux températures élevées et aux températures basses.

Ces recherches ont montré que *T. mossambica* s'acclimate très rapidement à des témperatures élevées et que la température maximum fatale est un peu au dessus de 38°C. D'un autre coté, l'acclimatation aux températures basses est très lente et on n'a pas mesuré à date une température minimum fatale. Cette acclimation lente aux basses températures est aussi liée à une grande susceptibilité à certaines températures (entre 8° et 15°C) selon la concentration totale des sels dissous ou de la pression osmotique de l'eau dans ces conditions. Des estimés de "LD_{50}" basés sur des températures qui amènent le coma du refroidissement secondaire indiquent clairement que la température de l'eau des barrages durant l'hiver est le facteur responsable des mortalités nombreuses. Ceci est tout spécialement clair chez les individus de petite taille (12 cm.) de cette espèce des cichlides.

EXPERIMENTELLE ANALYSE DER FAKTOREN, WELCHE FÜR DAS PERIODISCHE STERBEN VON TILAPIA MOSSAMBICA PETERS IN DEN BUSHVELD STAUSEEN IM TRANSVAAL VERANTWORTLICH SIND. B. R. Allanson, M. Ernst, und R. G. Noble. p. 293 .
Die Ursache der periodischen Fischsterben in den Stauseen des Bushveld - Highveld Übergangsgebietes im Transvaal, Südafrika wurde in den Laboratorien des National Institute for Water Research und des Department of Nature Conservation, Transvaal Provincial Administration experimentell untersucht.

Viele dieser Stauseen erhalten Abwässer von Kläranlagen und Industrien. Diese beiden Ursachen sind für das periodische Sterben, besonders von *Tilapia mossambica* verantwortlich gemacht worden. Aber die Tatsache, dass diese Fischsterben während der katen trockenen Winter haufiger sind, lässt vermuten, dass die niedrigere Wassertemperatur im Winter eine wichtigere Rolle als die Einleitung von Abwässern in die Stauseen der Dämme spielt. Deshalb wurde die Toleranz und der Widerstand von *T. mossambica* gegen hohe und niedrige Temperaturen untersucht.

Diese Studien zeigten, dass *T. mossambica* fähig ist, sich sehr rasch auf hohe Temperaturen einzustellen und dass die obere Tödlichkeitsgrenze etwas über 38° C. liegt. Auf der anderen Seite ist die Einstellung auf niedere Temperaturen sehr langsam und bisher konnte noch keine allerletzte niedrigere tödliche Temperatur gemessen werden. Dieses langsame Einstellen auf niedere Temperaturen steht auch im Zusammenhang mit der hohen Empfindlichkeit für diese Temperaturen (zwischen 8 und 15°C.) und hängt ausserdem von der Konzentration der gelösten Salze oder vom osmotischen Druck der Lösung ab. LD_{50} Bestimmungen auf Grund der Temperaturen, welche sekundäres Kältecoma hervorrufen,zeigen deutlich, dass die Wassertemperatur in diesen Stauseen im Winter für die zahlreichen Todesfälle, besonders der Grössengruppe von etwa 12 cm. dieser Cichlidenart, verantwortlich ist.

THE ENVIRONMENTAL REQUIREMENTS OF CENTRARCHIDS WITH SPECIAL REFERENCE TO LARGEMOUTH BASS, SMALLMOUTH BASS, AND SPOTTED BASS. George Bennett. p. 156 . It is impossible to define exact habitat requirements for any species of centrarchid bass. As with most species of fish, the embryos and yolk sac fry are usually much more vulnerable than are adults or advanced fingerlings to unfavorable physical, chemical, or biological conditions in the habitat. Laboratory experiments indicate that largemouth fingerlings can withstand sudden changes in pH, and excessively high and relatively low levels of dissolved oxygen. The absence of bass in an aquatic habitat may indicate unfavorable physiochemical conditions, but more often it is an indication of unfavorable or abnormal ecological interrelationships.

LES BESOINS DE MILIEU DE *CENTRARCHIDAE* AVEC RÉFÉRENCE SPECIAL DES *MICROPTERUS SALMOIDES, MICROPTERUS DOLOMIEUI,* ET DES *MICROPTERUS PUNCTULATUS.* George Bennett. p. 156. Il est impossible de définir les besoins exacts d'habitat pour aucune espèce des *Centrarchidae.* Pour le plupart des espèces de poissons, les embryons et les oeux sont ordinairement plus vulnérables que les adultes avancés, dans les conditions physiques, chimiques au biologiques défavorables de l' habitat. Les essais de laboratoire indiquent que les types de saumoneaux *Micropterus salmoides* peuvent résister en changements soudain de pH, et aux niveaux d'oxygène dégagée excèssivement haut et relativement bas. L'absence des *Centrarchidae* dans un habitat aquatique peut indiquer des conditions physico-chimiques défavorables mais bien sauvent c'est une indication de rapport écologique défavorable ou anormal.

DIE ANFORDERUNGEN DER CENTRARCHIDEN AN IHRE UMGEBUNG MIT BESONDERER BERÜCKSICHTIGUNG GEWISSER BARSCHE. George Bennett. p. 156
Die Arbeit berücksichtigt besonders Largemouth Bass, *Micropterus salmoides* Lacépède, Smallmouth Bass, Micropterus dolomieui Lacépède und Spotted Bass, *Micropterus punctulatus* Rafinesque.

Es erscheint unmöglich, die Anforderungen irgendeiner Art der centrarchiden Barsche an ihre Umgebung genau festzulegen. Wie es bei den meisten Fischarten der Fall ist, so sind auch hier Embryonen und frühe Entwicklungsstufen gegen ungünstige physikalische, chemische oder biologische Umweltbedingungen gewöhnlich empfindlicher als ältere oder erwachsene Tiere. Laboratoriumsversuche zeigen, dass junge *M. salmoides* plötzliche pH Änderungen und übermässig hohe wie auch verhältnismässig niedrige Konzentrationen von gelöstem Sauerstoff auszuhalten vermögen. Die Abwesenheit von Barschen in einem Gewässer kann ungünstige physikalisch-chemische Bedingungen anzeigen, viel öfter aber ist sie ein Hinweis auf ungünstige oder abnormale ökologische Verhältnisse.

EFFECTS OF TIME AND TEMPERATURE ON THE TOXICITY OF HEPTACHLOR AND KEPONE TO REDEAR SUNFISH. W.R. Bridges. p. 247 . The toxic effects of heptachlor and kepone were measured by the determination of the Median Effective Concentration (EC_{50}), the concentration required to produce 50 percent mortality of the fish at each time and temperature tested. EC_{50} values for heptachlor ranged from 0.017 milligram per liter for 96 hours' exposure at 75°F to 0.092 milligram per liter for 6 hours exposure at 45°F; comparable values for kepone were 0.034 milligram per liter and 5.6 milligrams per liter. The toxicity of kepone greatly increased with the time of exposure, whereas the influence of increased time on the toxicity of heptachlor was only moderate. Higher temperatures caused a moderate increase in toxic effects of both compounds.

LES EFFETS DE TEMPS ET DE TEMPÉRATURE SUR LA TOXICITÉ D'HEPTACHLOR ET DE KÉPONE A *LEPOMIS MICROLOPHUS.* W. R. Bridges. p. 247 . Les effets toxiques d'héptachlore et képone étaient mesurés par la détermination de la concentration effective médiane au EC_{50} (concentration éxigée pour produire 50% de mortalité des poissons) à chaque essais de temps et de température. Les valeurs EC_{50} pour l'héptachlore varient de 0.017 milligramme per litre pour 96 heures d'exposition à 27°C.; a 0.092 milligramme per litre pour 24 heures d'exposition a 7°C.; les valeurs comparables pour le képone étaient 0.044 milligramme per litre et 0.62 milligramme per litre. La toxicité de képone a beaucoup augmentee avec le temps d'exposition, tandis que l'influence de la longueur du temps sur la toxicité était seulement moderée. Des temperatures plus hautes ont seulement pour effet une légère augmentation en effets toxiques pour les deux composants.

EINFLUSS VON ZEIT UND TEMPERATUR AUF DIE GIFTIGKEIT VON HEPTACHLOR UND KEPONE GEGENÜBER REDEAR SUNFISH. W. R. Bridge. p. 247 . Die Giftwirkungen von Heptachlor (1,4,5,6, 7, 8, 8-hepta - chlor-3x, 4, 7, 7x-tetrahydro-4, 7-methanoinden) und Kepone (Deka - chloroktahydro-1, 3, 4-methano-2 H-cyklobuta CD - pentalen-2-on) wurden durch Bestimmung der mittleren wirksamen Konzentration (EC_{50}) gemessen, nämlich jener Konzentration, welche 50% Sterblichkeit des Redear Sunfish *(Lepomis microlophus)* bie der jeweiligen Versuchszeit und Temperatur verursacht. EC_{50} Werte für Heptachlor schwankten zwischen 0,017 mg/l fur 96 Stunden Versuchszeit bei 75° F (etwa 24°C) und 0,092 mg/l fur 6 Stunden Versuchszeit bei 45°F (etwa 8°C); die entsprechenden Werte für Kepone waren 0,034 mg/l und 5,6 mg/l. Für längere Versuchszeiten war die Giftigkeit von Kepone bedeutend grösser als aus den Ergebnissen von kurzen Versuchszeiten zu erwarten war, während der Einfluss längerer Versuchszeiten auf die Giftigkeit von Heptachlor nur mässig war. Höhere Versuchstemperaturen verursachten geringe Zunahme in der Giftwirkung beider Verbindungen.

THE BIOLOGY OF THE TUBIFICIDAE WITH SPECIAL REFERENCE TO POLLUTION. R.O. Brinkhurst. p. 57 . The title of this paper is a little ambitious in relation to our knowledge of the biology of the aquatic oligochaetes and of the Tubificidae in particular. The subject is reviewed, however, under three headings: the establishment of physical and chemical tolerance limits for individual species; the search for indicator species whose mere presence or absence can be used to categorize the water concerned; and detailed analyses of community structure, emphasizing identification to species. Our fragmentary knowledge under the first heading is reviewed, but few if any conclusions can be drawn as yet in view of the interaction of factors in nature, the variability of tolerance levels within the species in respect to different stages in the life cycle, and other complications. Under the second heading it is claimed that there is no such universal indicator organism available, certainly not in the Tubificidae. Finally, the pattern of species distribution in relation to known sources of pollution in British rivers is described, and the hope is expressed that detailed surveys along such lines will clarify this pattern and so render invertebrate surveys more complete and more useful as a diagnostic tool.

LA BIOLOGIE DES TUBIFEX SPÉCIALEMENT EN RAPPORT À LA POLLUTION. R. O. Brinkhurst p. 57 . Le titre de cet article semble un peu prétentieux quant à notre connaissance de la biologie des oligochètes aquatiques et des Tubifex en particulier. Toutefois le sujet est examiné de nouveau sous trois aspects: (1) établissement des limites de tolerance physique et chimique pour certaines espèces; (2) la recherche pour trouver des espèces indicatrices dont la présence ou l'absence suffirait pour définir la qualite de l'eau en question; (3) des analyses detaillées de la structure des populations en insistant sur l'identification des espèces. Nos connaissances restreintes du premier aspect font le sujet d'un examen détaillé, mais à date il est difficile d'arriver à des conclusions d'abord à cause de l'action combinée de facteurs dans la nature, puis de la variabilité des niveaux de tolérance pour une même espèce, à l'égard des stages différents du cycle vital et aussi à cause d'autres complications. Quant au deuxième point considéré, on croit qu'il n'existe aucun organisme indicateur universel de ce genre, du moins certainement pas chez les Tubifex. Pour finir on décrit la structure de la distribution des espèces par rapport aux sources de pollution dans les rivières d'Angleterre. On espère que des relevés détaillés dans le même sens pourront rendre cette structure plus plaire et par le fait même rendre ces relevés plus complets et plus utiles comme instrument diagnostique.

DIE BIOLOGIE DER TUBIFICIDAE MIT BESONDERER BERÜCKSICHTIGUNG VON WASSERVERUNREINIGUNGEN. Ralph Owen Brinkhurst. p. 57 . Die Überschrift dieser Abhandlung ist etwas ehrgeizig mit Bezug auf unser Wissen über die Biologie der Wasseroligochaeten und besonders der Tubificiden. Der Gegenstand wird unter drei Gesichtspunkten abgehandelt; die Festlegung physikalischer und chemischer Erträglichkeitsgrenzen für die einzelnen Arten, das Suchen nach Leitorganismen, deren Vorkommen oder Abwesenheit zur Beschreibung des betreffenden Wassers verwendet werden könnte und schliesslich genaue Analysen der zusammenvorkommenden Organismen mit besonderer Betonung der Identifizierung der gefundenen Arten. Unsere lückenhaften Kenntnisse des ersten Punktes werden erörtert, aber wegen der Wechselwirkung der einzelnen Faktoren in der Natur, wegen der Veränderlichkeit der Erträglichkeitsstufen innerhalb der einzelnen Arten während der verschiedenen Lebensabschnitte und wegen anderer Schwierigkeiten können gegewärtig kaum wichtige Schlüsse gezogen werden. Zum zweiten Punkt wird gesagt, dass z.Zt. kein allgemein brauchbarer Leitorganismus zur Verfügung steht, wenigstens nicht unter den Tubificiden. Schliesslich wird die Artenverteilung im Bezug auf bekannte Ursachen der Verunreinigung britischer Flüsse beschrieben und die Hoffnung ausgesprochen, dass ins Einzelne gehende Untersuchungen in dieser Richtung dieses Bild klären und dadurch Untersuchungen mit Wirbellosen zu einem brauchbaren diagnostichen Werkzeug machen werden.

ENVIRONMENTAL REQUIREMENTS OF SHRIMP. A.C. Broad. p. 86 . This paper is largely a review of an abundant literature that deals with three species of penaeid shrimp, *Penaeus setiferus*, *Penaeus aztecus*, and *Penaeus duorarum*, of direct commercial value in the United States. Although studies of these shrimp embrace a period of about 50 years and present a wealth of information on general ecology, life histories, distribution, and systematics, basic questions of the relationship of shrimp to their environment have not been answered or, indeed, dealt with in a manner likely to yield answers. Thus, while almost every investigator during the past 30 years has considered salinity in relation to shrimp distribution (including the author of this paper), it is still not possible to state what effect salinity may have, for example,

CONDITIONS DE MILIEU POUR LES CREVETTES. A. C. Broad. p. 86 . Cette communication est principalement une revue de l'abondante littérature concernant trois espèces de crevettes (bouquets), de valeur commerciale aux Etats Unis: *Penaeus setiferus*, *Penaeus axtecus*, et *Penaeus duorarum*. Quoique les études sur ces crevettes couvrent une période de 50 ans et présentent une richesse d'information sur leur écologie générale, leur histoire, leur distribution et la systématique, les questions de base sur les relations entre les crevettes et leur milieu n'ont pes été résolues ou du moins traitées d'une manière susceptible de donner une réponse. Ainsi, tandis que la plupart des chercheurs pendant les 30 dernières années (y compris l'auteur de ce papier) ont considéré le salinité en liaison avec la distribution des crevettes, il n'est

UMWELTBEDINGUNGEN FÜR GARNELEN. A. C. Broad. p. 86 . Der vorliegende Bericht ist grossenteils eine Besprechung der reichen Literatur über drei Arten von penaeiden Garnelen, *Penaeus setiferus*, *Penaeus aztecus* und *Penaeus duorarum*, die in den Vereinigten Staaten wirtschaftlich wichtig sind. Obwohl solche Studien sich über einen Zeitraum von etwa 50 Jahren erstrecken und viel über allgemeine Oekologie, über Leben, Verteilung und Systematik enthalten, so bleiben doch grundlegende Fragen über die Beziehungen der Garnelen zu ihrer Umwelt unbeantwortet oder wurden in einer Weise angegangen, dass Antworten unwahrscheinlich sind. So hat während der verflossenen 30 Jahre fast jeder Forscher, einschliesslich des Verfassers dieses Berichtes, den Salzgehalt in seiner Beziehung

on the growth of shrimp. Indeed, there is no general agreement on the growth rates of the three species. Effects of temperature on shrimp are little known and possible effects of oxygen concentration are not known at all. Studies involving common pollutants have not been attempted.

Commercially harvested shrimp spawn at sea. Unlike the majority of crustacea that provide some measure of brood pouch protection, penaeids shed their eggs, evidently while swimming at some intermediate depth. The young hatch in the naupliar stage and pass through a long and complex larval phase at sea. The recently metamorphosed, postlarval shrimp usually are found in shallow estuaries of relatively low salinity and generally high temperatures. A direct relationship between salinity and the size of juvenile shrimp is demonstrable during the seaward migration of young. Some evidence indicates differences in salinity optima between the three commercial species, presumably effective during the juvenile phase that is adjudged the most critical period in the life history. Recent trends in the commercial fishery suggest a change in the species composition of the penaeid population of the waters adjacent to the southeastern United States. The significance of this is obscure.

The need for basic physiological research on commercially harvested shrimp is considered to be acute.

pas encore possible d'établir quel effet peut avoir la salinité sur, par exemple, la croissance des crevettes. En vérité, il n'existe aucun accord général sur la vitesse de croissance de ces trois espèces. L'influence de la température est peu connue et celle possible de la concentration en oxygène dissous ne l'est pas du tout. Des études sur les substances polluantes communes n'ont pas été abordées.

Les crevettes récoltées commercialement frayent en mer. Contrairement à la majorité des crustacés qui assurent quelque protection à leur poche d'oeufs, les pénéides perdent leurs oeufs en nageant à des profondeurs moyennes. Le jeune à l'éclosion se trouve au stade nauplius et passe par une longue phase larvaire complexe en mer. Les crevettes post-larvaires, nounouvellement métamorphosées, se trouvent généralement dans les estuaires peu profonds, de salinité relativement faible et à des températures élevées.

Une relation directe entre la salinité et la teille de la jeune crevette est perciptible pendant la migration des jeunes vers la mer. Quelques remarques indiquent des différences dans l'optimum de salinité entre les trois espèces commerciales, effectives sans doute pendant la phase juvénile qui est jugée la plus critique de toute la vie. De nouvelles orientations dans la pêche commerciale suggérent un changement dans la composition des espèces de la population pénéide des eaux adjacentes aux Etats Unis du Sud Est. La signification de ceci est obscure.

La nécessité d'une recherche physiologique de base sur les crevettes récoltées commercialement apparait urgente.

zum Vorkommen der Garnelen studiert; und doch kann der Einfluss des Salzgehalts z. B. auf das Wachstum der Garnelen auch heute noch nicht angegeben werden. Sogar über die Wachstumsraten der drei Arten sind die Meinungen noch nicht einheitlich. Die Einflüsse der Temperatur sind wenig bekannt und über die möglichen Wirkungen der Sauerstoffkonzentration weiss man so gut wie nichts. Studien im Zusammenhang mit den gewöhnlich anzutreffenden Verunreinigungen sind noch nicht versucht worden.

Die Garnelen des Handels laichen im Meer. Im Gegensatz zur Mehrheit der Crustaceen, die ihren Jungen zu einem gewissen Grade Schutz in einem Beutel bieten, verbreiten die Penaeiden ihre Eier anscheinend während sie in mittlerer Tiefe schwimmen. Nach dem Nauplienstadium machen die Jungen eine lange und verwickelte Larvenentwicklung im Meer durch. Kurz darauf findet man die jungen Garnelen in seichten Flussmündungen von relativ geringem Salzgehalt und im allgemeinen hohen Temperaturen. Eine unmittelbare Beziehung zwischen Salzgehalt und Grösse der jungen Garnelen kann während ihrer Wanderung ins Meer beobachtet werden. Anzeichen sind vorhanden, dass Unterschiede in den Salzgehaltbestwerten für die drei handelsüblichen Arten bestehen, die vermutlich während des Jugendstadiums, das allgemein als die kritische Zeit im Leben dieser Tiere angesehen wird, wirksam sind. Seit einiger Zeit bestehen in der Handelsfischerei Hinweise darauf, dass eine Änderung der Artenverteilung in den Penaeidenvorkommen der Küstengewässer der südöstlichen Vereinigten Staaten feststellbar ist. Die Bedeutung dieser Beobachtung ist nicht klar.

Grundlegende physiologische Forschungen an Garnelen des Handels werden als dringend nötig erachtet.

ROLE OF WATERSHED MANAGEMENT IN THE MAINTENANCE OF SUITABLE ENVIRONMENTS FOR AQUATIC LIFE. W.E. Bullard. p. 265. This paper defines "watershed," "watershed management," and "suitable environments for aquatic life"; describes the many causes of change in the environment; and tells how the factors in change may be controlled or modified. Natural causes of change cited are earth movements, lightning, fires, reworking of stream channels by major floods, and animals. Development of a water-

LE RÔLE DE CONDUITE DU LIGNE DE PARTAGE DES EAUX POUR MAINTENIR LES MILIEUX CONVENABLE POUR LA VIE AQUITIQUE. W. E. Bullard. p. 265 . Cette communication définie «ligne de partage des eaux,» «administration de le ligne de partage des eaux», et «les milieux convenables pour le vie aquatique» et décrit les causes multiples de changement dans le milieu, et expose comment les facteurs en changement peuvent être commandés ou modifiés. Les causes de changement naturel citées sont les mouve-

DIE ROLLE DER ÜBERWACHUNG VON EINZUGSGEBIETEN ZWECKS AUFRECHTERHALTUNG EINER FÜR DAS LEBEN IM WASSER GÜNSTIGEN UMGEBUNG. William E. Bullard, Jr. p. 265 . Der Artikel definiert „Wasserscheide oder Einzugsgebiet", „Einzugsgebietkontrolle" und „für das Leben im Wasser geeignete Umgebung"; die mannigfachen Änderungen in der Umgebung werden beschrieben und es wird angegeben, wie diese Faktoren selbst wiederum gehandhabt, gesteuert oder geändert werden können. Natürliche Ursachen von Veränderungen

shed causes change through soil disturbance with consequent increased runoff, erosion, and sedimentation; through loss of shade by removal of the plant cover; through direct channel changes; and through additions of many kinds of materials to stream loads. Contributing factors subject to control include road construction, timber harvest, livestock grazing, farming, mining, miscellaneous construction, recreation, and waste disposal. Examples of recommended control methods are given. To control natural factors, animal populations can be modified, excess debris can be cleaned from channels, fires can be suppressed, flood control structures can be installed, and degraded watershed soil and cover can be rehabilitated.

ments de terre, feux d'éclair, le retraçage des canaux des fleuves par déluges majeurs,et les animaux. Le developpement d'une ligne de pertage des eaux cause des changements à travers le dérangement des sols avec pour conséquence, l'érosion, et la sédimentation; à travers la perte d'ombre par la suppression des plantes superficielles à travers le changement des canaux et à travers l'addition de beaucoup de types de limon. Le sujet de facteur de contribution par le contrôle incluent la construction de routes, le boisement, pâturages pour animaux domeatiqes, fermage, minage, constructions diverses, nouvelles creations et vastes dispositifs. Des exemples de controle recommendés sont donnés. Pour contrôler les facteurs naturels, les populations animales peuvent êntre mofifié, les excès de debris peuvent etré nettoyé des canaux, les feux peuvent être supprimé, le contrôle des structures des cours d'eau peut être installé et le sol dégradé et couvert peut être réhabilité.

sind Erdbewegungen, Feuer durch Blitzschlag,Verlegung von Flussläufen nach grösseren Überschwemmungen und Tiere. Die Entwicklung eines Einzugsgebietes verursacht Bodenveränderungen und als Folge vermehrten Abfluss, Erosion und Ablagerung; Entfernung der Pflanzendecke bringt Verlust von Schatten; Flussbettänderungen finden statt und vielartige Stoffe vermehren die Flussbelastung. Weitere Faktoren, welche kontrolliert werden können, sind Strassenbau, Holzfällung, Viehweiden, landwirtschaftliche Bodennutzung, Bergbau, Bauten verschiedenster Art, Wassersport und Abwasserbeseitigung. Als Beispiele fur die Kontrolle natürlicher Faktoren werden genannt Änderung der Tierwelt, Reinigung der Flussläufe, Unterdrückung von Feuern, Anlegen von I Hochwasserdeichen und die Verbesserung von Boden und Bodendecke schlechter Einzugsgebiete.

THE PROBLEMS IN DETERMINATION OF THE CAUSE OF FISH KILLS. G.E. Burdick. p. 289. Fish kills usually result from a spill or over-normal discharge of a pollutant that, after dilution, temporarily exceeds the tolerance of the species present. For this reason samples for analysis should be collected as soon as possible after the kill. Time-concentration toxicity curves covering 0 to 24 hours aid in evaluation.

Oxygen deficiency is the most frequent and most easily demonstrated cause of kill.

Mortality caused by toxic compounds from industrial operations is more difficult to prove. Basic information enabling chemical determination of compounds and decomposition products and of their possible effect and interaction is necessary.

Agricultural and pesticide chemicals, while usually not as difficult to evaluate, present major problems in chemical identification and quantitative determination due to extremely high toxicity. Present methods are not adequate to determine certain chemicals in fish tissue.

Interactions may require laboratory determinations on a specific water, under controlled oxygen content, pH, temperature, and concen-

PROBLEMES POUR DÉTERMINER LES CAUSES DE MORTALITÉ DES POISSONS. G.E. Burdick. p. 289. Les mortalités de poissons sont dues en général à un rejet au à un déversement anormal de substances polluantes qui, après dilution, dépassent momentanément la tolérance des espèces présentes. Pour cette raison, les échantillons pour analyses devraient être prélevés aussitôt que possible après la mortalité. Les courbes do toxicité, temps en function de la concentration, pendant 24 h., aident à l'estimation.

Un manque d'oxygène est la cause de mortalité la plus fréquente et la plus facile à prouver.

Une mortalité provoquée par des composés toxiques provenant d'opérations industrielles est plus difficile à démontrer. Il est nécessaire de posséder des informations de base permettant la détermination chimique des composés et des produts de décomposition, leurs effets possibles et leurs inter-actions.

Les produits chimiques agricoles et pesticides, généralement moins difficile à évaluer, présentent de grands problèmes d'identification chimique et de dosage quantitatif en raison de leur très grande toxicité. Les méthodes actuelles ne permettent pas de doser certains produits chimiques dans les tissus des poissons.

DIE PROBLEME IN DER FESTSTELLUNG DER URSACHEN VON FISCHSTERBEN. George E. Burdick. p. 289. Fischsterben sind gewöhnlich die Folgen eines Überfalls oder einer übernormalen Einleitung einer Verunreinigung, welche nach Verdünnung zeitweise die Toleranz der vorhandenen Arten überschreitet. Deshalb müssen Analysenproben so bald als möglich nach dem Sterben gesammelt werden. Giftigkeitsschaubilder, in welchen die Konzentration gegen die Zeit von 0 bis 24 Stunden aufgetragen ist, helfen in der Beurteilung.

Sauerstoffmangel ist die häufigste und am leichtesten feststellbare Ursache eines Fischsterbens. Sterblichkeit infolge von Giftstoffen aus industrieller Tätigkeit ist schwieriger nachweisbar. Die chemische Bestimmung von Verbindungen und Zersetzungsprodukten, sowie ihrer Wirkung und Wechselwirkung erfordern grundlegende Kenntnisse der möglicherweise in Betracht kommenden Industrie.

Landwirtschaftliche Chemikalien und Ungeziefervertilgungsmittel sind ein grösseres Problem in der chemischen Identifizierung und quantitativen Bestimmung, obwohl sie gewöhnlich unschwer bestimmt werden konnen. Unsere heutigen Methoden sind für die Bestimmung gewisser Chemikalien im Fischgewebe unzureichend.

trations of possible synergists or antagonists corresponding to those present at the time of kill. Confirmation by experimentally altered solution may also be necessary.

The greatest needs are for more exact and sensitive chemical procedures, a system of screening chemical compounds, improved information of synergism and antagonism procedures for utilizing post mortem analysis, and mathematical models permitting evaluation and correction for simultaneous interaction between components of the toxic system.

REACTION OF ESTUARINE MOLLUSKS TO SOME ENVIRONMENTAL FACTORS. Philip A. Butler. p. 92. This history of oyster culture demonstrates that within broad limits these mollusks survive and even prosper over a wide range of environmental conditions. Experience also demonstrates that apparently subtle, or at least for the most part unidentified, environmental factors may drastically alter the character and quality of oysters. A study to delineate some of the causal factors has been conducted for more than a decade on a natural population of oysters in Santa Rosa Sound, an estuarine environment in northwest Florida. The population, too small to be fished commercially, is a complex of three native species of the genera *Ostrea* and *Crassostrea*.

Continuous records have been maintained on seasonal and annual changes in salinity, temperature, rainfall, and the set of oysters on experimental cultch. Comparative data have been collected and are summarized showing the relationships of water pumping rates, shell movement, and growth to such features as water currents, food (chlorophyll), and carbohydrate-like substances normally present in the environment.

The sensitivity of shell deposition as a criterion of well-being of the oyster is described, and data are presented summarizing the response of various mollusks to artificial pollutants, such as agricultural pesticides, under laboratory con-

Les inter-actions peuvent demander des essais de laboratoire réalisés avec une eau spécifique, une concentration d'oxygène, un pH et une température contrôlée et des concentrations en éléments antagonistes au synergiques possibles, correspondant à celles présentées au moment de la mortalité.

Une confirmation par une solution poullée expérimentalement peut aussi être nécessaire.

Il serait très nécessaire d'avoir des méthodes chimiques plus exactes et plus sensibles, un système de triage des composés chimiques, de meilleures informations sur les processus de synergisme et d'antagonisme ofin de les utiliser dans les analyses post mortem, et des tables mathématiques permettant l'évaluation et la correction pour des inter-actions simultanées entre les composants d'un système toxique.

RÉACTION DE MOLLUSQUES D'ESTURINE À DES FACTEURS DU MILIEU AMBIANT. Philip A. Butler. p. 92 . Ce rapport de culture d'huitres, démontre que dons de vastes limites, ces collusques survivent et même prospèrent dans un large domaine de condition de milieu ambient. L'expérience démontre aussi qu-apparamment subtil, ou au moins pour la plupart non identifié, les facteurs du milieux ambiant peuvent alterer rigoureusement le caractère et la qualité des huitres. Une étude pour délimiter quelques un des facteurs accidentels, a été faite pour plus d'une décade sur une population naturelle d'huitres dans Santa Rosa Sound, un milieu estuairin dans le Nord Ouest de la Floride. La population trop petite pour être péché commercialement est un complexe de trois espèces des genera *Ostrea* et *Crossastrea*.

Les mentions continuelles ont été relevé sur les changements saisoniers et annuels en salinité, température, chute de pluie et sur le jeu d'huitre de culture expérimentale. Les donnees comparatives ont été collecté et sont resumées, montrant les relations entre le taux d'eau pompé, le mouvement des coquilles, la croissance de telles characteristiques et les courants de l'eau, la nourriture (chllorophyle), la substance comme hydrate de carbone normalement présente dans le milieu.

La sensitivité de déposition de coquille comme critère de bien-être de l'huitre est décrit et les

Wechselwirkungen können Laboratoriumsbestimmungen an einem bestimmten Wasser unter kontrollierten Bedingungen bezüglich Sauerstoffmenge, pH, Temperatur und Konzentrationen möglicherweise vorhandener synergistisch oder antagonistisch wirkender Substanzen erfordern, welche den zur Zeit des Fischsterbens vorhandenen entsprechen. Bestätigung durch geänderte Versuchslösungen kann ebenfalls nötig sein.

Am nötigsten sind genauere und empfindlichere chemische Verfahren, ein System zum Aussondern chemischer Stoffe, bessere Kenntnisse über Synergismus und Antagonismus zur Anwendung in post mortem Auswertungen und mathematische Modelle, welche die Bestimmung und Verbesserung für gleichzeitige Wechselwirkungen der Bestandteile des giftigen Systems ermöglichen.

DIE REAKTION VON MOLLUSKEN AN FLUSSMÜNDUNGEN GEGENÜBER EINIGEN UMGEBUNGSFAKTOREN. Phillip A. Butler. p. 92 . Die Geschichte der Austernkultur zeigt, dass diese Mollusken innerhalb weiter Grenzen zu leben vermögen und dass sie trotz grosser Unterschiede in den Umgebungsbedingungen wohl gedeihen. Die Erfahrung lehrt auch, dass anscheinend geringfügige oder wenigstens grösstenteils nicht identifizierte Umgebungsbedingungen den Charakter und die Güte der Austern drastisch ändern können. In einer Studie, die sich über mehr als 10 Jahre erstreckte und an einer natürlichen Austernansiedlung im Santa Rosa Sund, einer Flussmündung in Nordwestflorida, durchgeführt wurde, wird versucht, diese zufälligen Faktoren zu schildern. Die Ansiedlung war zu klein, um geschäftlich ausgebeutet zu werden; sie umfasste eine Gruppe von drei einheimischen Arten der Gattungen *Ostrea* und *Crassostrea*.

Jahreszeitliche und jährliche Änderungen des Salzgehaltes, der Temperatur, des Regenfalls und des Ansetzens der Austern an Versuchsmaterial wurden dauernd aufgezeichnet. Vergleichbare Werte wurden gesammelt und in einer Übersicht zusammengefasst; diese zeigt die Beziehungen zwischen Wasserförderleistung, Schalenbewegung und Wachstum auf der einen Seite zu solchen Faktoren wie Wasserströmung, Nahrung (Chlorophyll) und den gewöhnlich in dieser Umgebung vorhandenen kohlenhydratähnlichen Stoffen auf der anderen.

ditions. The data indicate the differential reaction of oysters to these pollutants and stress the need for continuing research to select effective agricultural and marine pest control chemicals that are relatively nontoxic to desirable estuarine forms.

données sont présentées resumant dans des conditions la réaction de laboratories de mollusque variés aux polluants artificiels tel que pesticides agricoles. La donnée indique les différents réactions des huitres à ces polluants et donne le moyen de continuer la recherche pour un contrôle de pesticide marins et agricoles qui sont relativement non toxiques.

Die Empfindlichkeit der Schalenablagerung als des Anzeigers der Gesundheit der Austern wird beschrieben und Versuchsergebnisse werden gebracht, welche die Reaktion verschiedener Mollusken auf künstliche Verunreinigungen, wie landwirtschaftliche Schädlingsvertilgungsmittel, unter Laboratoriumsbedingungen zusammenfassen. Die Ergbnisse zeigen die verschiedenartigen Reaktionen der Austern diesen Verunreinigungen gegenüber und betonen die Notwendigkeit für mehr Forschungsarbeit, damit wirksame chemische Stoffe zur Schädlingsbekämpfung in Landwirtschaft und Fischerei gewählt werden können, welche für wünschenswerte lebende Formen an Flussmündungen verhältnismässig ungiftig sind.

THE ENVIRONMENTAL REQUIREMENTS OF FRESH WATER PROTOZOA. John Cairns, Jr. p. 48 . Although free-living Protozoa have a cosmopolitan distribution, a particular species will occur only where the appropriate environmental conditions exist. Since the environment changes constantly, a constant replacement of the component species of a protozoan population is a normal event. An examination of protozoan populations from 202 areas, classified as healthy or semi-healthy according to the system of Patrick (1949), in rivers and streams of the United States and other parts of the world resulted in identification of nearly 1200 species. Approximately 75 percent of these species occurred in three or fewer areas, or less than 1.6 percent, of the areas sampled. Of the 25 percent that occurred four of more times, only 20 species were found in 25 percent of the areas studied. For those species with saprobian designation, some sampling areas had excellent agreement in composition, although the greater number did not. An association matrix was made for the 20 most common species. A Chi square test of significance was run on the 190 possible associations of species pairs, and of these, 44 pairs occurred together more frequently than would be expected from chance alone at the 5 percent level of confidence. The data indicated that associations of three or more species also existed. Three main facts were noted in the course of the analysis: (1) pairs or larger groups of associated species always had virtually identical ranges of environmental conditions; (2) these species always tolerated rather broad ranges of environmental conditions; and (3) having identical ranges of tolerance

LES BESOINS DE MILLIEU POUR LES PROTOZOAIRES D'EAU DOUCE. John Cairns, Jr. p. 48 . Bien que les protozoaires à l'état libre aient une distribution cosmopolite, des espèces particulières se présenteront seulement où les conditions convenables de milieu existent. Puisque le milieu change constamment, un remplacement continuel des espèces constituant une population de protozoaires, est un évenément normal. Un examen des populations de protozoaires de 202 zones, classées comme saines out semi-saines d'après le système de Partick (1949), dans les fleuves, les rivieres des États-Unis et les autres parties du monde, donne une identification de près de 1200 espèces. Approximativement 75% de ces espèces se presentaient dans trois zones et même moins, soit moins de 1.6% des zones échantillonnées. Des 25% qui se presentait quatre fois ou plus, seulement vingt espèces furent trouvées dans 25% des zones étudiées. Pour ces espèces avec les désignations saprobian certaines des zones d'échantillons avaient d'excellent rapport en composition, malgré que le plus grand nombre n'y soit pos. Une matrice d'association fut faite pour les vingt espèces, les plus communes. Une chi carré épreuve de signification fut faite sur les 190 associations de paires des espèces possibles, et 44 des 190 paires se presentèrent ensemble plus frequemment que ne le donnait le facteur de chance, ce facteur de chance aurait été donné a 5%. Les donneés ont indiqué que les associations de trois espèces ou plus ont existé également. Trois faits principaux marquaient dans le cours de l'analyse. (1) Les paires des plus grands groupes des espèces ont toujours eu des domaines presque

DIE ERFORDERNISSE VON FRISCHWASSERPROTOZOEN HINSICHTLICH IHRER UMGEBUNG. John Cairns, Jr. p. 48 . Obwohl freilebende Protozoen über die ganze Welt verbreitet sind, so werden doch die einzelnen Arten nur dort auftreten, wo geeignete Umgebungsbedingungen bestehen. Da sich die Umgebung dauernd ändert, so ist der ständige Ersatz einer bestimmten Protozoenart in einer Ansiedlung ein normales Ereignis. Eine Prüfung von Protozoenvorkommen an 202 Fundstellen in Flüssen und Strömen der Vereinigten Staaten und anderer Länder führten zur Identifizierung von nahezu 1 200 Arten. Die Fundstellen konnten nach dem System von Patrick (1949) als „gesund" oder „halb - gesund" bezeichnet werden. Ungefähr 75% der verschiedenen Arten traten in 3 oder weniger Fundorten, also in weniger als 1,6% der Beobachtungsstellen, auf. Von den 25%, welche viermal oder öfters vorkamen, wurden nur 20 Arten in 25% der untersuchten Gebiete gefunden. Für die als Fäulnisbewohner bezeichneten Arten zeigten einige Beobachtungsstellen sehr gute Übereinstimmung in der Zusammensetzung; für die Mehrheit traf das jedoch nicht zu. Eine Assoziationsmatrix wurde für die 20 am häufigsten auftretenden Arten gemacht. Eine X^2 Probe wurde für die 190 möglichen Assoziationen von Artenpaaren gemacht und von diesen kamen 44 Paare zusammen häufiger vor als durch Zufall allein auf der 5% Stufe der Zuverlässigkeit zu erwarten gewesen wäre. Die Werte zeigten, dass Assoziationen von drei oder mehr Arten ebenfalls vorkamen. In der Hauptsache wurden drei Tatsachen im Laufe der Analyse beobachtet: (1) Paare oder grössere Gruppen von zusammen auftretenden Arten hatten stets praktisch die to the chemical and physical environment does not insure that species will be associated more often than would happen by chance alone.

Due to the relatively low number of associated species and the broad range of tolerance of these species to the chemical and physical environment, it is evident that the best citerion for evaluation of the degree to which the requirements of Protozoan populations are being met is the diversity of species within these populations. Since Protozoa are part of a larger aquatic community, the entire aquatic flora and fauna should first be evaluated and the structure and diversity of the protozoan population analyzed both as a unit and in terms of its relationship to the structure of the larger community of which it is a part.

identiques aux conditions de milieu; (2) Ces espèces ont toujours toleré les domaines plûtot vaste des conditions de milieu; et (3) avoir les domaines de tolérance identique au milieu chimique et physique, n'assure pas que les espèces seront associées plus souvent qu'elles ne le seraient par hasard. Vue le nombre relativement bas des espèces associées et le large domaine de tolerance de ces espèces, au milieux chimique et physique it est évident que les meilleurs critères pour l'évaluation du degré dans lequel les besoins de protozoaires sont requis, est la diversite des espèces à l'intérieur même de ces populations. Pisque les protozoaires occupent la plus grande partie de la communauté aquatique, la faume et la flore aquatique seraient permièrement évaluées, la structure et la diversité des populations protozoaires analysées en bloc et en ces termes, de sa relation avec la structure de la plus grande communauté à laquelle il appartient.

gleichen Bereiche der Umgebungsbedingungen, (2) diese Arten vertrugen immer ziemlich weite Bereiche deiser Bedingungen und (3) Arten, welche die gleichen Bereiche der Toleranz gegenüber chemischen und physikalischen Umgebungsbedingungen haben,, kommen nicht notwendigerweise öfter assoziiert vor als das durch Zufall allein der Fall wäre.

Infolge der relativ geringen Zahl der assoziierten Arten und wegen der breiten Bereiche der Toleranz für die chemische und physikalische Umgebung ist es klar, dass die beste Kennziffer zur Auswertung des Grades, bis zu welchem die Ansprüche von Protozoenvorkommen befriedigt werden, die Verschiedenheit dieser Arten innerhalb dieser Vorkommen ist. Da die Protozoen ein Teil einer grösseren Gemeinschaft sind, so sollte die gesamte Wasserflora und Fauna zuerst untersucht werden. Dann sollte der Aufbau und die Verschiedenheit der Protozoenvorkommen sowohl als Einheit wie auch hinsichtlich ihrer Beziehungen zum Aufbau der grösseren Gemeinschaft, dessen Teil sie sind, analysiert werden.

PESTICIDE-WILDLIFE RELATIONS. Oliver B. Cope. p. 245. This subject covers the length and breadth of most approaches to the pesticide problem as it relates to wildlife. Detection and/or measurement of organic pesticides in wildlife and the environment are needed to achieve an understanding of the patterns of toxicity. The methods to be used for detection and measurement depend upon the objective of the study, the availability of assay methods, and the capability of the laboratory. Toxicity to aquatic life can be understood and predicted only with a knowledge of the effects of a toxicant on the environment, including food; the influences of factors in the environment on toxicity; the relative roles of chronic and acute toxicity; and a comprehensive characterization of the individual animals under consideration.

LES RELATIONS PESTICIDES SUR LA VIE SAUVAGE. Oliver B. Cope. p. 245. Ce sujet couvre la totalité de la plupart des accès au problème des pesticides en vie. La decouverte et, ou la mesure des organiques pesticides en vie, et le milieu, sont employés pour la complète compréhension des exemples de toxique. Les méthodes à utiliser pour la découverte et la mesure dépendent de l'objectif de l'étude, de la disponsibilité des méthodes d'épreuve, et de la capacité du laboratoire. La toxiquité pour la vie aquatique peut être comprise et prédite seulement avec la connaissance des effects d'un toxique, comprenant: la nourriture, l'influence des facteurs dans le milieu de la toxiquité, les rôles relatifs de toxiquité chronique et aigue, et une caractérisation compréhensive de l'animal individuel en considération.

DIE BEZIEHUNGEN ZWISCHEN INSEKTENVERTILGUNGSMITTELN UND NATURSCHUTZ. Oliver B. Cope. p. 245. Der Gegenstand umfasst in den verschiedensten Richtungen die meisten Wege zur Frage der Ungeziefervertilgungsmittel in ihrer Beziehung zum Naturschutz. Entdeckung und Messung von organischen Ungeziefervertilgungsmitteln in Organismen in ihrer natürlichen Umgebung sind zum Verständnis der Giftigkeitsbilder nötig. Die zu diesem Zweck benützten Verfahren hängen vom Ziel der Untersuchung, von der Zuverlässigkeit der Prüfungsmethoden und von der Fähigkeit des Laboratoriums ab. Giftigkeit gegenüber im Wasser lebenden Organismen kann nur dann verstanden und vorausgesagt werden, wenn die Wirkungen eines Giftstoffes auf Umgebung und Nahrung, der Einfluss gewisser Umweltfaktoren auf die Giftigkeit, die relative Rolle chronischer und akuter Giftigkiet und eine umfassende Beschreibung der Einzeltiere bekannt sind.

THE EFFECT OF ENVIRONMENTAL FACTORS ON CRAB DEVELOPMENT. John D. Costlow, Jr. p. 77 . Development of the *Brachyura* generally follows a similar pattern: a series of stages of free-swimming larvae, which through successive molts attain an intermediate or transitional form, which in turn metamorphose to the pre-adult. Techniques have recently been developed for rearing crab larvae under controlled laboratory conditions, from hatching to the first crab stage and beyond. The availability of large numbers of larvae of known stage of development and age enabled us to investigate many phases of larval development which previously had been impossible or impractical with larvae obtained from the plankton.

Experiments thus far have been primarily concerned with the effects of three or four environmental factors on larval development. These have included studies on the individual and combined effects of salinity, temperature, light, and diet and how these factors affect length of individual larval stages, the total time required for development to the crab, the number of larval stages, and survival at individual stages as well as the over-all survival. Of the 20 species that have been successfully reared to date four or five are cited as examples of how environmental factors can affect larval development.

Salinity tolerance varies considerably from species to species as well as with different larval stages of the same species. All larval stages of *Rithropanopeus harrisii* complete their development at low salinities (5 to 15 ppt) but have poor survival at higher salinities. Larvae of the driftline crab, *Sesarma cinereum*, do not develop in salinities lower than 20 ppt, and the larvae of the commercial blue crab, *Callinectes sapidus*, survive best in salinities of from 30 to 33 ppt. Although the first three zoeal stages of *S. cinereum* survive at 31.1 ppt, the fourth stage zoeae rarely metamorphose to the megalops at this salinity. The highest percent survival of larvae of the mud-crab, *Panopeus herbstii*, however, was at this same salinity, 31.1 ppt. Temperature, in addition to affecting the speed of development, may alter the normal salinity tolerance of the individual larval stages.

Although photoperiod does not appear to affect the development of *Sesarma reticulatum* larvae, con-

INFLUENCE DU MILIEU SUR LE DEVELOPPEMENT DU CRUBE. John D. Costlow, Jr. p. 77 . La développement des *Brachyur* suit généralement un ordre identique: une série de stades larvaires à nage librex, qui, per mues successives attaignent une forme intermédiaire ou transitoire laquelle, à son tour, se métamorphose en pré-adulte. Des techniques ont récemment été développées pour élever des larves de crabes en laboratoire dans des conditions déterminées depuis l'éclosion jusqu'au premier stade crabe et au délà. La disponibilité d'un grand nombre de larves d'âge et de stade de développement connus nous a permis d'étudier de nombreuses phases de développement larvaire ce qui était impossible auparavant avec des larves recueillies dans le plancton.

Jusqu'à présent les expériences ont été orientées sur l'influence de 3 ou 4 facteurs du milieu sur le développement larvaire. Nous avons étudié notamment l'influence individuelle et combinée de la salinité, de la température, de la lumière et du régime alimentaire et comment ces facteurs influencent la durée de chaque stade larvaire, la durée nécessaire au développement complet du crabe, le nombre de stades larvaires et la survie aux differents stades aussi bien que la survie totale. Sur les 20 espèces qui ont pu être élevées avec succès jusqu'à ce jour, 4 au 5 sont citées pour montrer à titre d'exemple combien les facteurs du milieu environnant peuvent affecter le développement larvaire.

La tolérance à la salinité varie beaucoup d'une espèce à l'autre, aussi bien que d'un stade à l'autre pour une même espèce. Tous les stades larvaires de *Rithropanopeus harrisii* achèvent leur développement pour de faibles salinités (5 à 15 ‰) mais n'ont qu'une faible survie à de plus fortes salinités. Les larves de *Sesarma cinereum* ne se développent pas à des salinités inférieures à 20 ‰ et les larves de crabe bleu, *Callinectes sapidus* survivent mieux dans des salinitiés de 30 à 33 ‰. Quoique les trois premiers stades zoé de *S. cinereum* survivent à 31,1 ppt. le 4e stade zoe se métamorphose rarement en mégalope à cette salinité. Cependant le plus grand pourcentage de survie du crabe *Panopeus herbstii* se trouve à cette même salinité de 31,1 ‰. La température, en plus de son influence sur la vitesse de développement, peut altérer la tolérance normale à la salinité des différents stades larvaires.

DER EINFLUSS VON UMGEBUNGSFAKTOREN AUF DIE ENTWICKLUNG VON KRABBEN. John D. Costlow, Jr. p. 77 . Die Entwicklung der *Brachyura* folgt im allgemeinen den gleichen Linien: eine Reihe von freischwimmenden Larvenstufen, dann eine Übergangs - oder Zwischenstufe, welche schliesslich in die Jungendform übergeht. Seit kurzem sind Aufzuchtverfahren von Krabbenlarven bis zur ersten Krabbenstufe und darüber hinaus unter Laboratoriumsbedingungen bekannt. Wir hatten eine grosse Anzahl von Larven bekannter Entwicklungs - und Altersstufen und konnten deshalb viele Phasen der Larvenentwicklung studieren, was früher mit Larven aus Plankton unmöglich oder unpraktisch war.

Bisher beschäftigten sich die Versuche hauptsächlich mit den Wirkungen von drei oder vier Umgebungsfaktoren auf die Larvenentwicklung entweder allein oder in ihrem Zusammenwirken, nämlich Salzgehalt, Temperatur, Licht und Nahrung. Der Einfluss dieser Faktoren auf Länge der Larven, Gesamtzeit nötig bis zur Entwicklung zur Krabbe, Zahl der Larvenstufen und Überleben sowohl der einzelnen Entwicklungsstufen wie der ganzen Entwicklungsreihe wurden studiert. Bisher wurden 20 Arten erfolgreich aufgezogen; davon werden 4 oder 5 als Beispiele für den Zusammenhang zwischen Umgebungsfaktoren und Larvenentwicklung aufgeführt.

Die Erträglichkeit für Verschiedenheiten im Salzgehalt ist von Art zu Art und für die gleiche Art innerhalb der Larvenstufen sehr verschieden. Alle Larvenstufen von *Rithropanopeus harrisii* entwickeln sich bei niedrigen Salzgehalten (5 bis 15‰), überleben aber höhere Salzkonzentrationen nur schlecht. Larven von *Sesarma cincereum* entwickeln sich unter 20‰ Salzgehalt überhaupt nicht, die der blauen Krabbe, *Callinectes sapidus*, überleben Konzentrationen von 30 bis 33‰, am besten. Obwohl die ersten drei Zoeenstufen von *S. cinereum* 31,1‰, aushalten, metamorphosiert die 4. Stufe bei diesem Salzgehalt nur selten . Larven von *Panopeus herbstii* indessen überlebten gerade diesen Salzgehalt von 31,1‰ am besten. Die Temperaturverschienheiten beeinflussen die Entwicklungsgeschwindigkeiten, können aber auch die normale Salztoleranz der einzelnen Larvenstufen ändern.

Obwohl die Lichtperiode auf die Entwicklung von Larven von *Sesarma reticulatum* ohne Einfluss zu sein scheint, so sind doch mehr Versuchssiderably more data are needed to evaluate all aspects of the effect of light.

Diet, both natural and synthetic, varies considerably in affecting larval development. Phytoplankton retard the time of development of some species, while in other species they do not affect development.

When the effects of the natural environmental factors on larval development are better understood, it will be possible to consider crustacean larvae as bioassay animals for a wide variety of studies. The regular sequence of stages, the intermolt periods of known duration, and the extreme sensitivity of larval stages to toxic substances should provide an excellent "indicator" for the study of water quality and pollution.

Quoique la photopériode ne semble pas influencer le développement des larves de *Sesarma reticulatum*, des résultats beaucoup plus nombreux sont nécessaires pour apprécier tous les aspects de l'activité de la lumière.

Le régime alimentaire, à la fois naturel et synthétique, influence le développement larvaire de facon très variable. Le phytoplancton retarde le développement de quelques espèces et est sans influence pour d'autres.

Lorsque l'action des divers facteurs du milieu naturel sur le développement des larves sera mieux comprise, il sera possible d'envisager les larves de crustacés comme animoux tests poud de nombreuses études. La succession naturelle des différents stades, la durée séparant les mues étant connue et la grande sensibilité des stades larvaires aux substances toxiques, fourniraient un excellent indicateur pour l'étude de la qualité des eaux et des pollutions.

sdaten nötig, ehe alle Seiten der Lichtwirkung ausgewertet werden können.

Natürliche und synthetische Nahrung wirkt auf die Larvenentwicklung sehr verschiedenartig ein. Phytoplankton verzögert die Entwicklungszeit bei einigen Arten, ist aber bei anderen Arten ohne Wirkung.

Wenn der Einfluss der natürlichen Umgebung auf die Larvenentwicklung besser bekannt ist, dann wird es möglich sein, Crustaceenlarven für viele biologische Studien zu verwenden. Die regelmässige Aufeinanderfolge der Entwicklungsstufen, ihre bekannte Zeitdauer und die ausserordentlich grosse Empfindlichkeit der Larven gegen Gifte könnten einen ausgezeichneten Anzeiger für Wassergüte und Verunreinigung liefern.

A SURVEY OF ENVIRONMENTAL REQUIREMENTS FOR THE MIDGE (DIPTERA: TENDIPEDIDAE). LaVerne L. Curry. p. 127. A brief history of the taxonomy and the limnological importance of the family is given. When possible, synonymy is given for the important species to enable the researcher to relate his data to that of different geographical regions. Interpretations of environmental requirements for the more common species of the family are based primarily on the Michigan fauna. Evaluations are made for the Holarctic, Neotropical, and Ethiopian realms, however, when these data are available. The environmental conditions considered are oxygen, carbon dioxide, sulfides, sulfites, acids, some organics, pH, temperature, fiber deposits, and siltation. These are considered with respect to midge populations in lotic and lentic environments, as well as in the littoral and profundal regions of freshwater lakes. The maximum conditions tolerable to midge populations in these environments are given when known. Several general conclusions are drawn from the study. Field work indicates that no group of midges can be regarded of pollutional indicator organisms per se. The species *Tendipes (T.) riparius* (Meigen) (=*Chironomus riparius* Meigen) and *T. (T.) tentans* (Fabricius) (=*Chironomus tentans* Fabricius) could be considered as such, however. Some species in the family

UN RELEVÉ DES CONDITIONS DE MILIEU REQUISES POUR LES CHIRONOMIDES (DIPTERA: TENDIPEDIDAE). LaVerne L. Curry. p. 127. On donne ice un bref résumé de la toxonomie et de l'importance limnologique de cette famille. Autant que possible on donne une liste de synonymes pour les espèces importantes afin d'aider le chercheur à rapporter ses données à celles des régions géographiques différentes. Les interprétations des conditions de milieu requises pour les espèces les plus communes de cette famille sont basées principalement sur la faune du Michigan. Cependant on fait des évaluations pour les domaines holoarctiques néotropical, et éthiopien lorsque ces données sont accessibles. Les conditions de milieu considérées sont l'oxygène, l'anhydride carbonique, les sulfites, les acides (les uns organiques), le pH, la température, les dépôts fibreux, et l'envasement. Ces conditions sont étudiées en rapport avac les populations de diptères dans les lacs et les rivières ainsi que dans les régions littorales et profondes des lacs d'eau douce. Les conditions maxima que les populations de diptères peuvent tolérer dans ces milieux sont données ici quand elles sont conneus. On a tiré de cette étude plusieurs conclusions générales. Le travail sur le terrain indique qu'on ne peut se servir d'aucun groupe de diptère *per se* comme organisme indicateur de pollution. Les espèces *Tendipes (T.)*

EIN ÜBERBLICK ÜBER DIE ANFORDERUNGEN DER ZUCKMÜCKE (DIPTERA: TENDIPEDIDAE) AN IHRE UMGEBUNG. LaVerne L. Curry. p. 127. Es wird eine kurze Geschichte der Taxonomie und der limnologischen Wichtigkeit der Familie gegeben. Wenn möglich werden sinnverwandte Namen für die wichtigen Arten angegeben, damit der Forscher seine Ergebnisse mit denen aus anderen geographischen Gebieten verknüpfen kann. Die Darstellungen der Anforderungen der gewöhnlich vorkommenden Arten an ihre Umgebung fussen hauptsächlich auf der Fauna des Staates Michigan. Soweit Angaben vorhanden sind, werden die Auswertungen auf die holoarktischen, neotropischen und aethiopischen Gebiete ubertragen. Folgende Umgebungsbedingungen werden berücksichtigt: Sauerstoff, Kohlendioxyd, Sulfide, Sulfite, Sauren, einige organische Stoffe, pH, Temperatur, Ablagerungen von Fasern und Verschlickung. Dann werden auch im Zusammenhang mit Zuckmuckenvorkommen als Umgebungsfaktoren sowohl stehende als fliessende Gewässer und seichte und tiefe Zonen von Süsswasserseen in Betracht gezogen. Wenn bekannt, werden die jeweils besten Bedingungen für die Mücken genannt. Einige allgemeine Schlüsse können aus diesen Studien gezogen werdden. Beobachtungen an Ort und Stelle zeigten, dass keine Gruppe von Zuckmücken für sich allein als Leitorganismus für Ver-

are restricted either to a lentic or lotic environment. Others are known to inhabit both environments. In addition, a few species are able to adapt to the physical and chemical conditions imposed upon the organisms in the littoral and profundal regions of larger bodies of water. In general, the conditions discussed in this paper, when imposed upon the midge fauna, have a detrimental effect upon the population.

ACCUMULATION OF RADIONUCLIDES OF AQUATIC INSECTS. J. J. Davis. p. 211. The section of the Columbia River that is within the Hanford Reservation in Southeastern Washington provides a unique opportunity for studying accumulation and cycling of radionuclides in natural aquatic communities. Insects living in the river below reactor outfalls are many times more radioactive than the water they inhabit. An organism's location in the food web, its trophic level, may affect the amount of radionuclides accumulated. Pronounced differences may occur in the amounts of radionuclides accumulated by different species of insects from the same habitat. Radionuclide concentrations within a species, however, follow definite seasonal cycles. The concentration of radionuclides in plankton follows essentially the same seasonal pattern as the radionuclide cycle in river water. The radioactivity in insects, on the other hand, coincides closely to the water temperature pattern.

The influence of isotopic dilution upon phosphorus-32 accumulation in aquatic organisms was studied by introducing the trace amount of phosphorus-32 but differing quantities of stable phosphorus, into each of four troughs containing established stream-type ecosystems. The results indicated a nearly linear reduction of phosphorus-32 uptake with increase of phosphorus in the water.

riparius (Meigen) (=*Chironomus riparius* Meigen) et *T. (T.) tentans* (Fabricius) (=*Chironomus tentans* Fabricius) pourraient cepedant être considérées comme telles. Quelques espèces de cette famille sont restreintes soit aux lacs soit aux rivières. On sait que d'autres espèces habitent les deux milieux. De plus, quelques espèces peuvent s'adapter aux conditions physiques et chimiques imposées aux organismes dans les régions littorales et profondes des grandes masses d'eau. En général, les conditions étudiées dans ce travail, si on les impose à la faune de diptères, ont des effets nuisibles sur la population.

ACCUMULATION DES RADIONUCLIDES PAR LES INSECTES AQUATIQUES. J. J. Davis. p. 211. La section de la rivière Columbia qui se trouve dans les limites de la Réserve Hanford, dans le sud-est de l'État de Washington, présente une occasion unique d'étudier l'accumulation et les phénomènes cycliques des radionuclides dans les communautés aquatiques naturelles. Les insectes qui vivent dans la rivière en aval des exutoires des réacteurs ont une radioactivité beaucoup plus élevée que celle de l'eau qu'ils habitent. La place d'un organisme dans la chaîne alimentaire, son niveau trophique, pourrait offecter la quantité de radionuclides accumulés. On peut trouver des différences marquées dans les quantités de radionuclides accumulés par les espèces différentes d'un même habitat. Les concentrations de radionuclides pour une espèce donnée subissent toutefois des variations saisonnières définies. La concentration des radionuclides dans le plancton suit essentiellement les mêmes variations saisonnières que celles des radionuclides dans l'eau de rivière. D'un autre coté, la radioactivité chez les insectes et la température de l'eau montrent des variations semblables qui coïncident pratiquement.

Pour étudier l'influence de la dilution isotopique sur l'accumulation du phosphor-32 chez les organismes aquatiques, on a introduit une quantité marquée de phosphore-32, mais des quantités differentes de phosphor stable dans chacune des quatre auges contenant des écosystèmes établis du genre rivière. Les résultats ont indequé une diminution presque linéaire dans l'assimilation du phosphore-32 à mesure que le phosphore augmente dans l'eau.

unreinigung angesehen werden kann, dass aber die Arten *Tendipes (T.) riparius* (Meigen) = *Chironomus riparius* (Meigen) und *T. (T.) tentans* (Fabricius) = *Chironomus tentans* (Fabricius) als solche angesehen werden könnten. Einige Arten der Familie kommen entweder nur in stehenden oder in fliessenden Gewässern vor, während andere diese Unterscheidung nicht machen. Ausserdem können sich einige wenige Arten an die physikalischen und chemischen Bedingungen der seichten und tiefen Zonen grösserer Wasseransammlungen anpassen. Im allgemeinen hatten die Versuchsbedingungen, welche Gegenstand unserer Untersuchungen waren, einen nachteiligen Einfluss auf die Zuckmükkenfauna.

ANREICHERUNG VON RADIONUKLIDEN DURCH IM WASSER LEBENDE INSEKTEN. J. J. Davis. p. 211. Der Abschnitt des Columbiaflusses innerhalb des Hanford Sperrgebiets im südöstlichen Teil des Staates Washington bietet eine einmalige Gelegenheit zum Studium der Anreicherung und des Kreislaufs von Radionukliden in natürlichen Gewässern. Insekten, die im Fluss unterhalb des Reaktorausfalls leben, sind viel radioaktiver als das Wasser, das sie bewohnen. Die Stellung eines Organismus im Nahrungshaushalt der Natur und die Art seiner Nahrungsverwertung können den Betrag der angesammelten Radionuklide beeinflussen. Wesentliche Unterschiede dieser Beträge können für verschiedene Insektenarten, auch solchen aus der gleichen Umgebung, auftreten. Innerhalb derselben Art folgt die Radionuklidkonzentration bestimnten jahreszeitlichen Wechseln. Die Radionuklidkonzentration im Plankton folgt im allgemeinen dem gleichen jahreszeitlichen Bild wie der Radionuklidwechsel im Wasser des Flusses. Die Radioaktivität der Insekten, auf der andern Seite, fällt im wesentlichen mit dem Wassertemperaturgang zusammen.

Der Einfluss der Isotopenverdünnung auf die Phosphor - 32 Anreicherung in Wasserorganismen wurde studiert, indem eine Spur von Phosphor - 32, aber verschiedene Mengen von stabilem Phosphor, in jeden von 4 Trögen eingetragen wurden, welche bekannte oekologische Typen Flusswasser enthielten. Die Abnahme von Phosphor - 32 Anreicherung war mit zunehmender Phosphorkonzentration im Wasser nahezu gradlinig.

DISSOLVED OXYGEN REQUIREMENTS OF FISHES. Peter Doudoroff and Charles E. Warren. p. 145. Dissolved oxygen concentration has been shown to influence markedly the maximum or active rates of oxygen consumption and the sustained swimming performance capabilities of fresh-water fishes at any given temperature. When food supply is unrestricted in laboratory tests, oxygen concentration influences likewise the rates of food consumption and of growth, and under extreme conditions, the gross efficiency of food conversion. The rates of embryonic and larval development, the size of larvae at the times of hatching and of completion of yolk absorption, and so the efficiency of yolk utilization, also are dependent on oxygen concentration. The effects of reduced concentrations on embryonic development vary not only with temperature, but also with the velocity of the water which determines the rate of delivery of oxygen to the chorion surfaces. Marked reductions of active rates of oxygen consumption by fish, of maximum sustained swimming speeds, and of rates of development, food consumption, and growth have been observed upon reduction of oxygen concentration to levels only slightly below the air-saturation level. The appetite and the growth rates of fish can be greatly impaired by wide diurnal fluctuation of oxygen concentration. The impairment occurs even when the arithmetic or geometric mean oxygen concentrations, i.e., the properly weighted means of the alternating low and high concentrations to which the fish are exposed, fall within the range of most favorable constant concentrations and the duration of daily exposure to low concentrations is only one-third or one-half of the 24-hour day. Abnormally high concentrations well above the air-saturation levels also can be inhibitory. Prompt avoidance reactions of fishes to reduced oxygen concentrations well above those that are lethal or cause obvious distress have been observed under experimental conditions.

The ecological significance of all the above observations made in the laboratory is not yet clear. Oxygen concentrations avoided under some experimental conditions may not be avoided likewise under natural conditions. Swimming speeds and developmental rates that are essential to survival and unimpaired success of fish in natural environments, as well as larval sizes that must be attained at the time of hatching or of complete yolk absorption are un-

CONDITION D'OXYGÈNE DISSOUS DES POISSONS. Peter Doudoroff and Charles E. Warren. p. 145. La concentration d'oxygène dissous a été montré pour influencer les taux maximum ou actif de consommation d'oxygène et la libre évolution des poissons d'eau douce à des températures données. Quand l'alimentation en nourriture n'a aucune restriction dans les essais de laboratoires, la concentration d'oxygène influence de même les taux de consommation de nourriture et les taux de croissance et sous d'extrême conditions la grosse efficience de conversion de nourriture. Les taux de développement au niveau des embryons et des larves, le grandeurs des larves au moment de l'éclosion et de la complete absorpiton des oeufs et aussi de l'efficience de l'utilisation des oeufs sont aussi dependant de la concentration d'oxygène. Les effets de concentration réduit sur le développement embryonnaire varient non seulement avec la temperature mais aussi avec la vitesse de l'eau qui détermine le taux de délivrance d'oxygène aux surfaces. Les réductions marqués de taux actif de consommation d'oxygène par le poisson de vitesse d'évolution soutenue et de pourcentage de développement de consommation de nourriture et de croissance ont été observé sous la réduction de concentration d'oxygène à niveau légèrement plus bas que le niveau d'air saturé. L'appétit et le pourcentage de croissance du poisson peut être grandement altérés par de fluctuation diurne de concentration d'oxygène. Les altérations persistene même quand le moyen de concentration d'oxygène est arithmétique ou géométrique. Par exemple, les moyens proprement évalués dans l'alternance des concentrations hautes et basses à laquelle les poissons sont exposés, tombent dans le domaine des concentrations les plus favorable et la durée d'exposition journaliere aux basses concentrations est seulement le 1/3 ou la moitie des 24 heures du jour. Les hautes concentrations anormalement bien au-dessus des niveaux d'air saturé peuvent aussi être reprimées. Les réactions promptes du poisson pour réduire les concentrations d'oxygène de celles qui sont lethal ou cause de détresse evidente ont été observé dans des conditions expérimentales.

La signification écologique de toutes les observations faites ci-dessous dans le laboratoire n'est pas encore claire. Les concentrations d'oxygène évitées dans des conditions expérimentales ne

FISCHE UND IHR BEDARF AN GELÖSTEM SAUERSTOFF. Peter Doudoroff und Charles E. Warren. p. 145. Es ist bekannt, dass die Konzentration des gelösten Sauerstoffs beträchtlich den Höchst - und Aktivwert des Sauerstoffverbrauchs und die Dauerschwimmleistung von Süsswasserfischen bei jeder beliebigen Temperatur beeinflussen. Bei unbeschränkter Nahrungsversorgung in Laboratoriumsversuchen wirkt sich die Sauerstoffkonzentation auch auf Raten des Nahrungsverbrauchs und des Wachstums und unter Grenzbedingungen auf die Stoffwechselleistung selbst aus. Die Raten der Embryonal - und Larvenentwicklung, die Larvengrösse zur Zeit des Ausschlüpfens, die Vollständigkeit der Dotterabsorption und damit die Leitungsfähigkeit der Dotterausnützung hängen ebenfalls von der Sauerstoffkonzentration ab. Die Einflüsse geringerer Konzentrationen auf die Embryonalentwicklung ändern sich nicht nur mit der Temperatur, sondern auch mit der Wassergeschwindigkeit, das diese die Geschwindigkeit der Sauerstoffabgabe an die Choionoberflache bestimmt. Bedeutende Verminderungen der aktiven Raten des Sauerstoffverbrauchs der Fische, der höchsten Dauerschwimmgeschwingigkeiten, der Entwicklungsraten, des Nahrungsverbrauchs und des Wachstums wurden beobachtet, wenn die Sauerstoffkonzentration nur sehr wenig unter den Luft - Sättigungsstand herabgesetzt wurde. Appetit und Wachstums raten von Fischen können durch weite tägliche Schwankungen der Sauerstoffkonzentration sehr beeinträchtigt werden. Die Schädigung tritt sogar dann auf, wenn die arithmetischen oder geometrischen Mittel der Sauerstoffkonzentrationen (d.h. die richtig gewerteten Mittel der abwechselnd niedrigen und hohen Konzentrationen, welchen die Fische ausgesetzt sind) in die Reihe der günstigsten Dauerkonzentrationen fallen und die Fische nur für etwa ein Drittel oder die Hälfte des 24 - Stundentages solch niedrigen Konzentrationen ausgesetzt sind. Abnormale Konzentrationen, beträchtlich über den Luftsättigungswerten können ebenfalls hemmend wirken. Unverzügliche Ausweichreaktionen der Fische im Bereiche geringerer Sauerstoffkonzentrationen, auch wenn solche bestimmt oberhalb der tödlichen Konzentrationen lagen oder augenscheinlich Unbehagen verursachten, wurden unter den Bedingungen der Versuche beobachtet.

Die oekologische Bedeutung aller angeführten Beobachtungen ist noch nicht klar. Unter gewissen Versuchsknown, but presumably can be determined experimentally. The critical oxygen concentration for incipient impairment of growth under natural conditions probably depends on the availability of food, which may itself be the major factor limiting growth rates. This critical concentration may well depend also on the rate at which energy must be expended by the fish in seeking and capturing available food, while also escaping enemies, etc. The appetite of the fish or the activity that presumably is necessary for food procurement, or both, evidently must be reduced when available oxygen becomes insufficient for supporting all metabolic processes at normal levels. Accordingly, a satisfactory laboratory model for studies of the dissolved oxygen requirements of fish, directed toward reliable estimation of critical concentrations at which incipient impairment of growth would occur in a given natural environment, cannot be a very simple one. Evidently it must combine the food ration normally obtainable under favorable dissolved oxygen conditions in the natural environment with enforced activity approximately equivalent to the spontaneous and other activity that under the natural conditions would be essential for survival and normal feeding. This conclusion applies also to the study of other water-quality requirements of fishes.

peuvent être évitées de même dans des conditions naturelles. La vitesse d'évolution et les taux de développement qui sont essentiels pour la survie et la non altération du poisson dans des milieux naturels aussi bien que la grandeur des larves qui doit être atteinte à l'eclosion au la complète absorption des oeufs sont inconnue, mais peuvent être déterminé expérimentallement. La concentration d'oxygène pour l'altération de croissance dans des conditions naturelles dépend probablement de la disponibilité de la nourriture qui peut elles-meme etre le facteur majeur de limitation du taux de croissance. Cette concentration critique peut bien dependre aussi du taux d'énergie dépensé par le poisson pour capturer la nourriture et aussi échapper aux ennemis, etc. L'appétit du poisson ou l'activite qui probablement est nécessaire pour le procurement da la nourriture ou pour les deux évidemment peut être réduit quand l'oxygène disponsible devient insuffisant pour supporter tous les procédés metaboliques aux niveaux normaux. En conséquence un modèle de laboratoire satisfaisant pour les études de condition d'oxygène dissous par le poisson dirige vers l'estimation des concentrations critiques à laquelle l'alterations de la croissance surviedrait dans un milieu naturel donné ne peut être pas une très simple realisation. Évidemment, il peut combiner la ration de nourriture normalement obtenu sous des conditions d'oxygène dissous favorable dans le milieu naturel avec l'activité approximativement équivalente et autres activités qui sous les conditions naturelles seraient essentielles pour l'alimentation normale et la survie. Cette conclusion s'applique aussi à l'étude des autres conditions de qualité d'eau en rapport avec le poisson.

bedingungen wurden Sauerstoffkonzentrationen vermieden, die in der Natur nicht vermieden wurden. Schwimmgeschwindigkeiten, welche für das Überleben und den ungehinderten Erfolg der Fische in ihrer natürlichen Umgebung notwendig sind, Larvengrössen, welche zur Zeit des Ausschlüpfens erreicht werden müssen oder vollkommene Dotterabsorption sind unbekannt, vermutlich aber experimenteller Bestimmung zugänglich. Die kritische Sauerstoffkonzentration für beginnende Wachstumshemmung unter natürlichen Bedingungen hängt wahrscheinlich von der verfügbaren Nahrung ab, die selbst ein wichtiger Faktor in der Beschränkung der Wachstumsraten sein kann. Diese kritische Konzentration kann auch von dem Masse abhängen, in welchem Energie von den Fischen zum Suchen und Fangen der verfügbaren Nahrung oder zur Vermeidung von Feinden usw. aufgewendet wird. Der Appetit der Fische oder die Tätigkeit, vermutlich nötig zur Nahrungsbeschaffung, oder beide, müssen augenscheinlich verringert werden, wenn der verfügbare Sauerstoff zum Unterhalt aller Stoffwechselvorgänge in normalem Massstab ungenügend wird. Infolgedessen kann ein zufriedenstellendes Laboratoriumsmodell zum Studium des Bedarfs von Fischen an gelöstem Sauerstoff, das zuverlässig die kritischen Konzentrationen für beginnende Wachstumsschadigung in einer naturlichen Umgebung bestimmen liesse, nicht sehr einfach sein. Augenscheinlich müsste es die normal unter günstigen Konzentrationen gelösten Sauerstoffs erhaltliche Nahrungsmenge in der natürlichen Umgebung mit erzwungener Tätigkeit vereinigen, welch letztere ungefähr gleich der unter denselben Bedingungen für das Uberleben und die normale Nahrungsaufnahme nötigen Tätigkeit sein müsste. Dieser Schluss ist auch für das Studium anderer Anforderungen, welche Fische an die Güte des Wassers stellen, gültig.

EFFECTS OF OIL POLLUTION ON MIGRATORY BIRDS. Ray C. Erickson. p. 177. The loss of migratory birds due to oil pollution has been a perpetual problem in the management of this resource. Because of their attraction to or dependence upon aquatic environments, many migratory birds are subject to factors that adversely affect the habitability of these environments.

The oil pollution problem is most critical during the cold months, for at that time migrating birds pass through or concentrate upon the

INFLUENCE DE LA POLLUTION PAR LES HUILES SUR LES OISEAUX MIGRATEURS. Ray C. Erickson. p. 177. La perte d'oxieaux migrateurs par suite de pollution par des huiles a été un problème continu pour l'aménagement de ces ressources. En raison de leur attraction ou de leur dépendance vis à vis des milieux aquatiques, beaucoup d'oiseaux migrateurs dépendent de facteurs affectant défavorablement l'habitat de ces milieux.

Le problème de la pollution par les huiles est très critique pendant

EINFLUSS VON WASSERVERUNREINIGUNG MIT ÖL AUF ZUGVÖGEL. Ray C. Erickson. p. 177. Der Verlust von Zugvögeln durch Wasserverunreinigung mit Öl ist ein dauerndes Problem in der Betreuung diese Naturschatzes. Viele Zugvögel sind, weil sie durch Wasser angezogen werden oder davon abhängen, jenen Faktoren ausgesetzt, welche die Bewohnbarkeit dieser Umgebung ungünstig beeinflussen.

Die Frage der Verunreinigung mit Öl ist während der kalten Monate äusserst kritisch, weil dann waters or shorelines where oil may be spilled or to which it may drift, and death from exposure may be rapid. Although oil contamination may occur inland from time to time, relatively small numbers of migratory birds ordinarily are affected by it because fewer birds are exposed or because the quantities of oil involved are less. Along the shores of North America, expecially the Atlantic and Gulf coasts, migratory bird feeding and resting areas are subject to oil contamination from sea-going vessels and shore installations. Continuing oil pollution is encountered at a few locations, but the most spectacular losses usually have occurred as a result of the escape of oil from storm- and collision-damaged or sunken vessels along the coast.

All birds frequenting oil-polluted shores and waters may be subject to oil contamination. Just a few drops of oil coming in contact with the plumage may destroy its waterproof nature and subject the bird to exposure or, if the oil is ingested, may have toxic effects in the digestive tract. Birds appear to be attracted to oil, presumably because of the quieting effect of the oil on choppy waters. Thus, the adverse effects of even a small oil slick may be much more important than its relatively limited area might lead one to expect, and when the area is frequented by large numbers of migrating or wintering birds, heavy mortality may result.

The only complete remedy to the problem is elimination of all sources of oil that contaminate migratory bird habitats. Although zealous enforcement of the terms of the International Convention for the Prevention of Pollution of the Sea by Oil, 1954, and strict controls over oil spillage or discharge into coastal waters should substantially reduce this problem, a complete solution in the foreseeable future can hardly be expected. Accidental spillage over which there is little or no control undoubtedly will continue, and both unintentional and deliberate pollution will probably occur in spite of the strictest enforcement of protective measures. It is possible that research into this problem might reveal methods of repelling birds from oil-contaminated water. Until such techniques are developed, however, the only remedy consists of capturing, decontaminating, and rehabilitating the birds by laborious and time-consuming methods; the number of birds salvaged by these methods usually is small compared with the number that perish.

les mois froids car c'est le moment où lex oiseaux migrateurs passent ou bien se rassemblent sur lex eaux ou sur les rivages; de l'huile peut y être répandue ou y avoir été entrainée, de sorte que la mort peut être rapide. Quoiqu une contamination par des hules puisse se présenter de temps en temps sur terre, relativement peu d'oiseaux migrateurs en sont affectés, soit parceque peu d'oiseaux y sont exposés, soit parceque les quantites d'huile entraînées sont moindres. Le long des plages de l'Amérique du Nord, particulièrement des côtes de l'Altantique et de Gulfe, les zones de rivitaillement et de repos des oiseaux migrateurs sont sujettes aux contaminations par les hydrocarbures provenant à la fois des navires en circulation et des installations côtières. Une pollution permanente existe en quelques endroits, mais les mortalitès les plus spectaculaires ont eu lieu à la suite soit d'un orage avec collision et échappement d'huile, soit de maufrage de bateaux le long de la côte.

Tous les oiseaux fréquentant les rivages et les eaux polluées par des huiles peuvent être sujets à la contamination. Quelques gouttes d'huile seulement venant en contact avec le plumage de l'oiseau peuvent détruire son imperméabilité et exposer l'oiseau à l'huile ou, si l'huile est absorbée, peuvent avoir des effets toxiques sur l'appareil digestif. Les oiseaux semblent attirés par l'huile, sans doute en raison de l'effet calmant de l'huile sur les eaux agitées. Aussi les effets dangereux d'une nappe h'huile, même petite, peuvent être beaucoup plus importants que sa surface limitée ne paurrait le laisser supposer et, guand la région est fréquentée par de nombreaux oiseaux, une mortalité importante peut en résulter.

Le seul remède complet au problème est l'élimination de toutes sources d'huile pouvant contaminer les habitats des oiseaux migrateurs. Quoique l'application rigouseuse des termes de la Convention Internationale pour la protection de la mer contre la pollution par hydrocarbures de 1954 et des contrôle; stricts des déversements de'huile dans les eaux côtières doivent réduire sensiblement ce problème, une solution complète peut difficilement être espérée dans un avenir proche. Les écoulements accidentels qui ne peuvent être contrôlés ou très peu, continueront et des pollutions aussi bien involontaires que délibérées auront

die Zugvögel auf ihrer Wanderung sich auf den Gewässern oder an den Ufern ansammeln und dort verschüttetes oder angetriebenes Öl ihren raschen Tod zur Folge haben kann. Obwohl Verunreinigungen durch Öl von Zeit zu Zeit auch im Innern des Landes vorkommen, so sind doch dann gewöhnlich weniger Zugvögel dadurch geschädigt, weil die Zahl der Vögel geringer ist und weil auch die Ölmengen nicht so bedeutend sind. Längs der Küsten von Nordamerika, besonders am Atlantischen Ozean und im Golf von Mexiko, sind die von Zugvögeln benützten Futter - und Ruheplätze der Verunreinigung durch Öl von Seeschiffen und von gewissen Küstenanlagen unterworfen. Dauerverunreinigung durch Öl tritt an einigen Stellen auf; am meisten fallen jedoch gewöhnlich die Verluste auf, welche durch Ölverlust aus sturm - oder zusammenstossbeschädigten oder aus gesunkenen Schiffen herrühren.

Alle Vögel können sich mit Öl beschmutzen, wenn sie damit verunreinigte Küsten oder Gewässer aufsuchen. Einige wenige Tropfen Öl können die wasserabweisende Natur des Gefieders zerstören; mit der Nahrung aufgenommenes Öl kann Giftwirkungen im Verdauungskanal auslösen. Vögel scheinen durch Öl angelockt zu werden, möglicherweise wegen der glättenden Wirkung des Öls auf die Wasserfläche. So können die nachteiligen Wirkungen eines nur kleinen Ölflecks viel wichtiger sein als seine geringe Oberflächenausdehnung vermuten liesse und wenn die Gegend von einer grossen Anzahl von Zugvögeln oder überwinternden Vögeln besucht wird, dann kann eine hohe Sterblichkeit die Folge sein.

Das Problem der Verunreinigung durch Öl kann nur durch vollkommene Ausschaltung aller Ursachen erreicht werden. Obwohl peinlich genaue Befolgung der Regeln des Internationalen Übereinkommens zur Verhinderung der Verunreinigung des Meeres durch Öl vom Jahre 1954 und strenge Kontrolle über verschüttetes Öl oder dessen Einleitung in Küstengewässer das Problem bedeutend verringern, so kann doch eine vollständige Lösung nicht so bald erwartet werden. Zufälliges Verschütten kann nur wenig oder gar nicht verhindert werden und wird zweifellos fortbestehen und unabsichtliche oder absichtliche Verunreinigung wird wahrscheinlich trotz strengster Schutzmassnahmen vorkommen. Möglicherweise werden weitere Studien des Problems zu einem Verfahren führen, welches die Vögel von ölverunreinigten Ge-

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Dramatic and pathetic as pollution-caused losses may be, damage to the habitat may be even more lasting in its effects on wildlife populations. Toxic influences of oil on plants and animals that directly or indirectly are important to migratory birds may far outweigh observable bird losses. Habitats are vulnerable to pollution throughout the year rather than just a few months during the fall, winter, and spring. Little is known regarding the duration or over-all chemical, mechanical, or biological effects of the oily residues that continue to accumulate and blanket the bottom with sludge, or of methods that might be used to restore the contaminated areas to full productivity.

probablement lieu en dépit de la plus stricte application des mesures de protection. Il est possible que des recherches sur ce problème revèlent des méthodes permettant d'éloigner des oiseaux contaminés. Cependant, jusqu à ce que de telles techniques soient trouvées, le seul remède consiste à capturer, décontaminer et rétablir les oiseaux par des méthodes longues et laborieuses, le nombre d'oiseaux sauvés par ces moyens est normalement petit, comparé au nombre de ceux qui périssent.

Aussi dramatiques et pathétiques que puissent être les pertes causées par la pollution, les dommages portés à l'habitat peuvent être encore plus durables dans leurs effets sur la vie des animaux sauvages. Les influences toxiques de l'huile sur les plantes et lex animaux qui, directement ou indiractement, interviennent dans la vie des oiseaux migrateurs, peuvent dépasser de beaucoup les pertes visibles d'oixeaux. Les habitats sont vulvérables à la pollution toute l'année et non quelques mois d'hiver. On sait peu de choses sur la durée des effets chimiques, mécaniques ou bioligiques des résidus huileux qui s'accumulent et recouvrent les fonds avec la vase ou sur les méthodes permettant de remettre en état les zones contaminées.

wässern abstösst. Aber bis zur Entwicklung solcher Methoden besteht das einzige Heilmittel darin, die Vögel in anstrengenden und zeitraubenden Verfahren zu fangen, zu reinigen und dann wieder freizulassen; die Zahl der auf diese Weise geretteten Vögel ist gewöhnlich gering im Verhältnis zur Zahl der Verluste.

Dramatisch und erschütternd wie diese durch Verunreinigungen hervorgerufenen Verluste sein mögen, der Schaden an den von den Vögeln besuchten Gegenden dauert fort in seinem Einfluss. Giftwirkungen von Öl auf Pflanzen und Tiere, welche mittel - oder unmittelbar wichtig für Zugvögel sind, können die beobachtbaren Verluste an Vögeln weit überwiegen. Wohnplätze sind wahrend des ganzen Jahres empfindlich für Verunreinigungen und nicht nur im Herbst, Winter und Frühling. Man weiss nur sehr wenig über die Dauer der chemischen, mechanischen oder biologischen Gesamtwirkungen der Ölrückstände, welche sich fortwährend ansammeln und die Böden der Gewässer mit Schlamm bedecken oder über Verfahren, welche die verunreinigten Gebiete wieder zu voller Ertragsfähigkeit bringen könnten.

SOME REMARKS ON A NEW SAPROBIC SYSTEM. E. Fjerdingstad. p. 232 . A system of grouping to show the relationship of each species to pollution places organisms in four groups: (1) saprobiontic, those occurring in large numbers only in heavily polluted waters; (2) saprophilons, those occurring generally in polluted waters but which may occur elsewhere; (3) saproxenons, those generally not occurring in polluted waters; and (4) saprophobons, those not surviving in polluted waters. In the system emphasizing community aspects of organisms, the polysaprobic zone is divided into three zones and "coprozoic" designates the zone for undiluted faecal water. There are nine zones, each with its community of algae and other organisms.

DES REMARQUES SUR LE NOUVEAU SYSTÈME SAPROBIQUE. E. Fjerdingstad. p. 232. Un système de groupement en qautre groupes pour montret la relation de chaque espèce sur les organismes dans les places polluées. (1) Saprobiontic survenant en grands nombre dans les eaux fortement polluées. (2) Saprophobons survenant généralement en eaux polluées mais pouvant survenir en d'autres places. (3) Saproxenons ne survenant généralement pas en eaux polluées. (4) Saprophobons, ceux, ne survenant pas en eaux polluées. Dans le systeme insistant sur l'aspect de communaté des organismes, la zone «polysaprobic» est divisée en trois zones et «coprozoic» désigne la zone de l'eau faecale indiluée. Il y a neuf zones, chancune avec sa communaté d'algues et d'autres organismes.

EINIGE BEMERKUNGEN ÜBER EIN NEUES SAPROBIENSYSTEM. E. Fjerdingstad. p. 232. Ein Einteilungssystem, das die Verwandtschaft jeder Art zu Verunreinigungen zeigen soll, bringt die Organismen in vier Gruppen unter: 1) saprobiontisch, in grosser Zahl nur in stark verunreinigten Wässern vorkommend; 2) saprophil, kommen im allgemeinen in verunreinigten Wässern vor, werden aber auch anderswo gefunden; 3) saproxenone, treten im allgemeinen in verunreinigten Wässern nicht auf; 4) saprophobone, welche in verunreinigten Wässern nicht leben können. In diesem System, welches das gleichzeitige Auftreten verschiedener Organismen betont, wird die Polysaprobienzone in drei Zonen unterteilt und „koprozoisch" bezeichnet die Zone unverdünnten Fäkalwassers. Man unterscheidet im ganzen 9 Zonen, jede mit ihrer eigenen Gruppe von Algen und anderen Organismen.

THE IMPORTANCE OF EXTRACELLULAR PRODUCTS OF ALGAE IN THE AQUATIC ENVIRONMENT. G.E. Fogg. p. 34 . A variety of organic substances may be liberated into the medium from healthy algal cells in laboratory culture, and there is evidence that similar extracellular products are released, sometimes in relatively large amounts, in nature. These extracellular products may have various effects of biological significance in aquatic habitats, e.g., chelation of ions or enzymic decomposition of organic substances. The liberation of glycollic acid as an extracellular product of photosynthesis may be of particular impo tance. The presence of a low concentration of this substance in the medium has been found to be necessary for the growth of a planktonic *Chlorella*, and there are indications that dissolved glycollic acid may act as a substrate for heterotrophic growth of microorganisms in freshwater.

L'IMPORTANCE DES PRODUITS EXTRACELLULAIRES DES ALGUES DANS LE MILIEU AQUATIQUE. G. E. Fogg. p. 34 . Dans les cultures de laboratoire, des algues saines peuvent libérer dans leur milieu environnant toute une variété de substances organiques. La même chose se produit aussi dans la nature où des produits extracellulaires semblables peuvent être libérés, parfois en quantités relativement grandes. Ces produits extracellulaires peuvent avoir des effets de signification biologique variée sur les habitats, par example: chélation d'ions, ou la décomposition des substances organiques par les enzymes. La liberation d'acide glycollique comme produit extracellulaire de la photosynthèse peut avoir une importance toute particuliere. La présence d'une petite quantité de cette substance dans le milieu est necessaire à la croissance d'une *Chlorella* du plancton, et de plus il semble que l'acide glycollique dissous pourrait aussi servir de base à la croissance hétérotrophique des microorganismes dans l'eau douce.

DIE WICHTIGKEIT VON EXTRAZELLULAREN ALGENPRODUKTEN IM WASSER. Gordon Edward Fogg. p. 34 . Eine Vielheit von organischen Substanzen kann in einer Laboratoriumskultur von gesunden Algenzellen in ihre Umgebung abgegeben werden und man hat Beweise dafür, dass ähnliche extrazellulare Stoffe auch in der Natur, manchmal sogar in relativ grossen Mengen, ausgeschieden werden. Diese Stoffe können verschiedene Wirkungen von biologischer Bedeutung im Wasser haben, z. B. Chelation von Ionen oder enzymatischen Abbau organischer Stoffe. Das Freiwerden von Glykolsäure als ein extrazellulares Produkt der Photosynthese könnte von besonderer Wichtigkeit sein. Es wurde gefunden, dass eine niedere Konzentration dieser Säure für das Wachstum einer Plankton Chlorella notwendig ist und es scheint, dass Glykolsäure in Lösung als Substrat für das heterotrophe Wachstum von Mikroorganismen in Süsswasser wirkt.

RELATIONSHIPS BETWEEN THE CONCENTRATION OF RADIONUCLIDES IN COLUMBIA RIVER WATER AND FISH. R. F. Foster and Don McConnon. p. 216 . The relationship between the concentration of radionuclides in water and in fish used for food is a necessary consideration in the release of radioactive wastes to surface waters. Actual field observations provide good reference data and clues to the mechanisms involved in the transfer of the nuclides from the water to the fish. A few radionuclides are concentrated in fish to such an extent that human consumption of the fish is the factor that limits the release rate.

Downstream from the Hanford reactors, chromium-51, arsenic-76, and neptunium-239 are the most abundant nuclides in Columbia River water, but these are relatively unimportant in the fish. The biologically important radioelements, phosphorus-32 and zinc-65, are present in relatively low concentrations in the river, but are dominant in the fish. The uptake of radionuclides by fish can change markedly with the season of the year and the most pronounced variations occur with relatively short-lived nuclides acquired via a food chain. Wide

RELATIONS ENTRE LA CONCENTRATION DE RADIONUCLIDES DANS LE POISSON ET L'EAU DU FLEUVE COLUMBIA. R. F. Foster and Don McConnon. p. 216. La relation entre la concentration de radionuclides dans l'eau et le poisson utilisé pour le nourriture et une considération necessaire au sujet de la décharge de déchets radioactifs dans les eaux de surface. L'actuel champs d'observation produit de bonnes données de référence et un indice des mécanismes entrainés dans la transfert des nuclides de l'eau au poisson. Peu de radionuclides sont concentrés dans le poisson à tel point que la consommation humaine du poisson est le facteur qui limite le taux de décharge.

Descendant le fleuve a partir des réacteurs d'Hanford, chrome-51, arsenic-76, et neptunium-239 sont les nuclides les plus abondants dans l'eau de fleuve Columbia, mais ceux-ci sont relativement très peu important dans le poisson. Les radioéléments biologiquement importants, phosphore-32 et zinc-65, sont présents en une relative basse concentration dans la rivière mais sont dominants dans le poisson. L'aptitude pour absorber des radionuclides pour le poisson

BEZIEHUNGEN ZWISCHEN DER KONZENTRATION VON RADIONUKLIDEN IM WASSER UND IN DEN FISCHEN DES COLUMBIAFLUSSES. Richard F. Foster and Dan McConnon. p. 216 . Die Beziehung zwischen der Konzentration von Radionukliden im Wasser und in Fischen, die als Nahrungsmittel dienen, ist eine wichtige Erwägung hinsichtlich der Entleerung radioaktiver Abfallstoffe in Oberflächenwasser. Beobachtungen an Ort und Stelle geben gute Bezugsdaten und Hinweise auf den Mechanismus bei der Übertragung der Nuklide vom Wasser auf die Fische. Einige Radionuklide sind in Fischen so stark angehäuft, dass Fischgenuss durch den Menschen der die Entleerungen von radioaktiven Abfallstoffen begrenzende Faktor geworden ist.

Flussabwärts von den Hanford Reaktoren sind Chrom - 51, Arsen - 76 und Neptunium - 239 die häufigsten Nuklide im Columbiaflusswasser, aber diese drei sind verhältnismässig unwichtig für Fische. Die biologisch wichtigen Radioelemente Phosphor - 32 und Zink - 65 sind im Fluss in verhälnismässig geringen Konzentrationen vorhanden, aber vorherrschend in den Fischen. Die Aufnahme der Radionuklide variations between individual specimens necessitate large numbers of samples for valid interpretation.

PHYSIOLOGICAL CONSIDERATIONS IN STUDIES OF THE ACTION OF POLLUTANTS ON AQUATIC ANIMALS. Paul O. Fromm. p. 316. To assess properly the effects of water pollutants on aquatic animals, long-term studies should be carried out in which non-acutely lethal concentrations of toxicants are used. Specific physiological parameters of a chemical, a morphological, or a histopathological nature are suggested and examples given of their use. In addition, a more general approach to the assessment of the effect of pollutants on aquatic animals is discussed. This procedure involves measurement of the general metabolism (oxygen consumption) of assay animals and is based on the assumption that no matter what environmental stress is placed on an animal, it will be reflected in a change (increase or decrease) in normal metabolic rates. Advantages and disadvantages of the two approaches to the study of water pollution are discussed.

ENVIRONMENTAL REQUIREMENTS OF PLECOPTERA. Arden R. Gaufin. p. 105 . While Plecoptera (stoneflies) constitute one of the smaller orders of insects, they are nevertheless one of the most important groups found in many streams. In mountain streams, they are a principal source of food for trout. Brinck reported that streams in Sweden with dissolved oxygen concentrations below 40 percent saturation had no stoneflies. Because of their preference for clean, well-aerated water, they are becoming increasingly important to limnologists as indicators of clean water conditions in pollutional surveys.

Practically all of the work that has been done to date on the North American stoneflies has dealt with their taxonomy and morphology. Despite the work that has been done, the nymphs of over 50 percent of our North American species have not

change considérablement avec la saison de l'année. Les grandes variations entre les specimens individuels nécessitent un grand nombre d'échantillons pour une interprétation valide.

CONSIDÉRATIONS PHYSIOLOGIQUES DANS LES ÉTUDES DE L'ACTION DES POLLUANTS SUR LES ANIMAUX AQUATIQUES. Paul O. Fromm. p. 316 . Pour évaluer proprement les éffets des polluants de l'eau sur les animaux aquatique, des études à long termes seraient mis à exécution dans lequelles une concentration non aigue de toxiques est utilisé. Les paramètres physiologiques d'un produit chimique, une nature morphologique ou histopathologique sont suggérés et des exemples donnés de leur utilité. En addition une approche plus générale pour l'évaluation de l'effet des polluants sur les animaux aquatiques est discutée. Cette methode implique la mesure du métabolism général (consommation d'oxygène) des animaux d'essais et est basée sur l'hypothèse que si aucun milieu n'est placé avec un animal il sera refléchi dans un changement (croissant ou décroissant) en vitesses métaboliques normales. Les avantages et les désavantages des deux approches de l'étude de pollution de l'eau sont discutées.

CONDITION DE MILIEUX AMBIANT DES PLÉCOPTERA. Arden R. Gaufin. p. 105 . Tandis que les *Plécoptera* constituent un des plus petits genres d'insecte, ils sont néammoins un des plus importants groupes trouvé dans beaucoup de cours d'eau. Dans les cours d'eau de montagne ils sont une source principals de nourriture pour les truites. Brinck a rapporté que les cours d'eau en Suède avec des concentrations d'oxygene dissous en dessous de 40% de saturation n'avaient pas de *Plécoptera.* Du fait de leur préférence pour l'eau propre et bien oxygéné ils sont devenus de plus en plus important aux yeux des limnologistes, comme des indicateurs de condition d'eau propre dans les examens de pollution.

Pratiquement tous les travaux qui ont été fait sur les *Plecoptera* d'Amérique du Nord ont partie lié avec leur taxonomie et leur morphologie. Malgré le travail que a été fait, les nymphes de plus de

durch Fische kann mit der Jahreszeit bedeutend schwanken und die grössten Änderungen treten in Nukliden von kurzer Lebensdauer auf, welche mit dem Futter aufgenommen werden. Grosse Unterschiede zwischen Einzelproben erfordern für zuverlässige Deutung der Ergebnisse eine grosse Anzahl von Proben.

PHYSIOLOGISCHE ÜBERLEGUNGEN ZU STUDIEN DER WIRKUNG VON VERUNREINIGUNGEN AUF WASSERTIERE. Paul O. Fromm. p. 316. Wenn die Wirkungen von Verunreinigungen des Wassers auf im Wasser lebende Tiere richtig abgeschätzt werden sollen, dann müssen Versuche von langer Dauer mit nicht akut tödlichen Konzentrationen der Giftstoffe ausgeführt werden. Spezifische physiologische Kennzeichen chemischer, morphologischer oder histopathologischer Natur werden vorgeschlagen und Anwendungsbeispiele werden gegeben. Ausserdem wird eine allgemeinere Methode zur Abschätzung der Wirkung von Verunreinigungen auf Wassertiere besprochen. Diese Methode erfordert Messung des allgemeinen Stoffwechsels (Sauerstoffverbrauchs) von Versuchstieren und fusst auf der Annahme, dass jede Bürde, welche einem Tier von der Umgebung auferlegt wird, sich in einer Änderung (Vermehrung oder Verminderung) der normalen Stoffwechselrate zu erkennen gibt. Vor- und Nachteile dieser zwei Methoden des Studiums von Wasserverunreinigung werden besprochen.

UMWELTBEDINGUNGEN DER PLECOPTERA. Arden R. Gaufin. p. 105 .Wiewohl Plecoptera ("stoneflies", Frühlingsfliegen) eine der kleineren Insektenordnungen bilden, sind sie trotzdem eine der wichtigsten in Strömen auftretenden Gruppen. In Bergflüssen sind sie die Hauptnahrungsquelle für Forellen. Brink berichtete, dass Flüsse in Schweden mit einer Konzentration von gelöstem Sauerstoff unter 40% Sättigung keine Frühlingsfliegen hatten. Da sie klares gut belüftetes Wasser bevorzugen, so werden sie in zunehmendem Grade als Anzeiger für reines Wasser in Studien über Wasserverunreinigung wichtig.

Praktisch alle bisherigen Arbeiten an nordamerikanischen Frühbeen described. This is particularly true of our western species.

Research by Hynes, by Aubert, and by Brinck, in Europe, indicated that European stoneflies differ perhaps more in their ecology than they do taxonomically or morphologically. In view of this fact, detailed information as to the habitat requirements and physiological differences of the various species may be quite essential to stonefly taxonomists if they ever separate successfully the many yet unidentified forms.

Most of the ecological data that have been published concerning both European and North American stoneflies consist of general statements as to habitat preferences. Hynes mentioned five major factors as controlling the distribution of stoneflies in England, namely, movement of the water, altitude, substratum, drought, and possibilities of colonization. Brinck considered the following factors as being most important in determining the distribution of Swedish stoneflies: movement of the water, substratum, temperature, the amount of certain gases and compounds in the water, and the presence of food.

The author has found that such factors as temperature, water velocity, bottom type, chemical composition of the water, and abundance of food are very important in determining the distribution of stoneflies in streams of the Intermountain Area. In order to determine the environmental requirements of stoneflies more precisely than can be done under field conditions, studies have been conducted in artificial streams in the laboratory during the last 3 years. The objectives of these studies have been to determine the effects of low dissolved oxygen concentrations at various temperatures and stream velocities on the gross activity of stoneflies, and to determine the minimum dissolved oxygen concentrations at which exposure for a prolonged period of time can be endured without lethal effects.

Optimal water temperatures for the species of stoneflies tested to date have been found to be between 50° to 60°F. In this temperature range three species of stoneflies were exposed to oxygen concentrations as low as 3 ppm for 24 hours with no mortality. Sensitivity to low dissolved oxygen concentrations increased rapidly with an increase of temperature above 60°F.

50% de nos espèces d'Amérique du Nord n'ont pas été décrites. Ceci est particulierement vrai pour nos espèces de l'ouest.

Les recherches par Hynes, Aubert, et Brinck, en Europe, ont indiqué que les *Plécoptera* européennes differentes peut-être plus dans leur écologie qu'ils different taxonomiquement et morphologiquement. Vue ce fait, l'information détailleé comme les conditions d'habitat et les différences physiologiques des espèces variées, peut être tout à fait essentiel au *Plécoptera* taxonomiste si ils ne séparent jamais les formes encore non identifiées.

La plupart des données écologiques qui ont été publié concernant les *Plécoptera* européennes et Nord-Americaine consiste en des rapports généraux comme les préférences d'habitat. Hyne a mentionné cinq facteurs majeur contrôlant la distribution de *Plécoptera* en Angleterre, ce sont: la mouvement de l'eau, l'altitude, le substratum, la sécheresse, et la possibilité de colonisation. Brinck a considéré les facteurs suivants comme étant plus important en déterminant la distribution de *Plécoptera* suèdois le mouvement de l'eau, le substratum, le température, la quantité de gaz et de composants dans l'eau, et la présence de nourriture. L'auteur a trouvé que des facteurs tels que la température, la vitesse de l'eau, le type du fond, la composition chimique de l'eau, et l'abondance de la nourriture, sont très importants pour déterminer la distribution de *Plécoptera* dans les cours d'eau occupant des régions non montagneuses. Afin de déterminer les conditions de milieu des *Plecoptera* plus précisement qu'elles peuvent être données dans des conditions de terrain, des études ont été faites en cours d'eau artificiel en laboratoire durant les trois dernières années.

L'objectif de ces études a été de déterminer les effets de basses concentrations d'oxygène dissous à des températures variées et de la vitesse des cours d'eau sur la grosse activité de *Plecoptera* et de déterminer le minimum de concentration d'oxygène dissous à laquelle l'exposition, pour une période de temps prolongée peut être endurée sans effets léthals.

Les températures de l'eau pour les *Plécoptera* essayés ont été trouvé entre 50° et 70°F. (10° et 20°C.). Dans ce domaine de température, trois espèces de *Plécoptera* furent exposés une concentration d'oxy-

lingsfliegen beschäftigten sich mit ihrer Taxonomie und Morphologie. Trotz all dieser Bemühungen sind die Nymphen von über 50% der nordamerikanischen Arten noch nicht beschrieben worden. Dies trifft besonders für die im Westen vorkommenden Arten zu.

Studien von Hynes, von Aubert und von Brink in Europa zeigten, dass die europäischen Frühlingsfliegen sich von den “stoneflies” vielleicht mehr in ihrer Oekologie als taxonomisch oder morphologisch unterscheiden. In Anbetracht dieser Tatsache können Arbeiten über die Anforderungen dieser Insekten an ihre Umwelt und über physiologische Unterschiede der verschiedenen Arten für Forscher auf diesem Gebiet sehr wichtig sein, wenn sie die vielen bisher nicht identifizierten Formen ordnen wollen. Die meisten veröffentlichten Angaben über europäische und nordamerikanische Arten sind allgemein gehalten im Bezug auf Umweltbedingungen. Für England gibt Haynes fünf die Verteilung beeinflussende Punkte: Wasserbewegung, Meereshöhe, Bodenschicht, Dürre und Möglichkeiten der Kolonienbildung. Brink hält für schwedische Frühlingsfliegen die folgenden Faktoren für wichtig: Wasserbewegung, Bodenschicht, Temperatur, Gehalt des Wassers an gewissen Gasen und Verbindungen und Nahrung.

Der Vortragende hat gefunden, dass Temperatur, Wassergeschwindigkeit, Bodenschicht, chemische Zusammensetzung des Wassers und Nahrungsfülle für die Bestimmung der Verteilung der “stoneflies” in Flüssen der sog. Intermountain Area sehr wichtig sind. Um die Umweltbedingungen dieser Insekten noch klarer festzulegen als das an Ort und Stelle gemacht werden kann, wurden während der vergangenen drei Jahre Versuche an künstlichen Strömen im Laboratorium durchgeführt. Sie sollten den Einfluss niedriger Konzentrationen an gelöstem Sauerstoff bei verschiedenen Temperaturen und Wassergeschwindigkeiten auf das allgemeine Verhalten der “stoneflies” zeigen und die Mindestkonzentration an gelöstem Sauerstoff bestimmen lassen, welche selbst in langen Zeiträumen für die Insekten nicht tödlich ist.

Besttemperatur des Wassers für die “stonefly” Art, welche bisher untersucht wurde, ist zwischen 50° und 60° F. Bei dieser Temperatur waren für drei Arten Sauerstoffkonzentrationen von nicht mehr als 3 mg/l. für 24 Stunden erträglich. Die Empfindlichkeit gegen niedrige

TOXIC WATERBLOOMS OF BLUE-GREEN ALGAE. Paul R. Gorham. p. 37 . The unialgal culture of colonies of blue-green algae isolated from toxic and non-toxic waterblooms has provided new insights into the old problem of algal poisoning. Toxicity tests have been conducted on 24 blooms representing 4 dominant species and 52 strains representing 10 species. Toxic and non-toxic strains of *Microcystis aeruginosa* Kutz. emend. Elenkin and *Anabaena flos-aquae* (Lyngb.) de Breb. have been isolated from toxic and non-toxic blooms. So far, only non-toxic strains have been isolated of three other species suspected of toxicity: *Aphanizomenon flos-aquae* (L.) Ralfs., *Gloetrichia echinulata* (J. E. Smith) P. Richter, and *Coelosphaerium Kutzingianum* Nageli. Bacteria associated with waterblooms have been found to produce toxic factors, but these appear to play a secondary role.

Toxic *Microcystis* strains produce a fast-death factor (FDF), which kills laboratory animals and livestock when administered orally. Toxic *Anabaena* strains produce a very-fast-death factor (VFDF), which is also active when administered orally. *Microcystis* FDF is an endotoxin whose production is physiologically as well as genetically controlled. It is ordinarily released during senescence or decomposition of the cells. It has been isolated and identified as one of a group of five rather similar polypeptides. *Microcystis* FDF has an LD_{50} (IP, mice) of 0.5 milligrams per kilogram; freeze-dried *Microcystis* cells have an LD_{100} (oral, various animals) of 1 to 2 grams per kilogram. *Anabaena* VFDF may be an exotoxin. It has some properties that resemble those of *Microcystis* FDF.

The development of a toxic waterbloom depends on the favorable interaction of at least six variables: (1) presence and dominance of a toxic strain of alga; (2) size and composition of the associated bacterial flora; (3) conditions required for algal toxin production; (4) conditions required for aggregating large masses of algae near shore; (5) conditions required for the release of toxins without undue dilution or destruction; and (6) ingestion of

gène en dessous de trois p.p.m. pour 24 heures sans relever de mortalite. La sensibilité aux basses concentrations d'oxygene s'est accrue rapidement avec une accroissement de température au-dessus de 60°F. (16°C.).

TOXICITE DES FLORAISONS DES ALGUES BLEUES-VERTES. Paul R. Gorham. p. 37 . Des cultures uniformes de colonies d'algues bleues-vertes prélevées de floraisons toxiques et non toxiques ont éclairci le vieux problème d'empoisonnement par les algues. On a fait des tests de toxicité sur 24 floraisons représentant 4 espèces prédominantes et sur 52 cultures représentant 10 espèces. A partir de floraisons toxiques et non toxiques on a pu isoler des cultures toxiques et non toxiques de *Microcystis aeruginosa* Kütz. emend. Elenkin et *Anabaena flos-aquae* (Lyngb.) de Bréb. Jusqu'à date, on a pu isoler seulement des cultures non toxiques de trois espèces qu'on croit être toxiques; ce sont: *Aphanizomenon flos-aquae* (L.) Ralfs., *Gloeotrichia echinulata* (J. E. Smith) P. Richter, et *Coelosphaerium Kützingianum* Nägeli. Les bactéries associées aux fluoraisons peuvent produire des éléments toxiques, mais ceux-ci semblent jouer un role secondaire.

Les cultures toxiques de *Microcystis* ont un élément de mort rapide (« FDF ») qui amène la mort d'animaux de laboratoire et de ferme lorsque administré par voie buccale. Les cultures toxiques de *Anabaena* ont aussi un élément de mort très rapide (« VFDF ») lorsque administré par voie buccale. L'élément « FDF » de *Microcystis* est une endotoxine dont la production est contrôlée aussi bien au point de vue physiologique que génétique. D'ordinaire cette endotoxine est libérée durant le vieillissement ou la décomposition des cellules. On a pu l'isoler et l'identifier comme faisant partie d'un groupe de cinq polypeptides similaires. L'élément « FDF » de *Microcystis* a un LD_{50} (« IP », souris) de 0.5 milligrammes par kilogramme. Les cellules de *Microcystis* séchées-congelées ont un LD_{100} (par voie buccale, différents animaux) de 1 à 2 grammes per kilogramme. L'élément « VFDF » *d'Anabaena* peut être une exotoxine. Certaines de ces propriétés ressemblent à celles de *Microcystis*.

Le développement d'une floraison toxique dépend d'au moins six facteurs et de leurs actions combinées, elles sont énumérées ci-dessous: (1) la présence et la prédominance

Konzentrationen an gelöstem Sauerstoff nimmt für Temperaturen über 60° F. sehr rasch zu.

GIFTIGE WASSERBLÜTE BLAU - GRÜNER ALGEN. Paul R. Gorham. p. 37 . Reinkulturen von Kolonien blau - grüner Algen aus giftigen und ungiftigen Wasserblüten haben neue Einblicke in das alte Problem von Vergiftungen durch Algen erlaubt. Giftigkeitsversuche wurden an 24 Wasserblüten, welche 4 vorherrschende Arten und 52 Stämme von 10 Arten darstellten, gemacht. Giftige und ungiftige Stämme von *Microcystis aeruginosa* Kützing emend. Elenkin und *Anabaena flos-aquae* (Lyngbye) de Brébisson wurden von giftigen und ungiftigen Blüten isoliert. Bisher wurden von drei anderen Arten, welche giftigkeitverdächtig waren, nämlich *Aphanizomenon flos - aquae* (L.) Ralfs, *Gloeotrichia echinulata* (J. E. Smith und Sowerby) P. Richter und *Coelosphaerium Kützingianum* Nägeli, nur ungiftige Stämme isoliert. Zusammen mit Wasserblüten vorkommende Bakterien können giftige Stoffe erzeugen, doch scheinen diese nur eine untergeordnete Rolle zu spielen.

Giftige *Microcystis* Stämme erzeugen einen rasch tödlich wirkenden Faktor (FDF), der Laboratoriumstiere und landwirtschaftliche Nutztiere bei *per os* Verabreichung tötet. Giftige *Anabaena* Stämme bringen einen ebenfalls *per os* sehr rasch tödlich wirkenden Faktor (VFDF) hervor. *Microcystics* FDF ist ein Endotoxin, dessen Erzeugung physiologisch und genetisch kontrolliert ist. Es wird gewöhnlich während des Alterns oder der Zersetzung der Zellen abgesondert. Es wurde isoliert und als eines von 5 einander ziemlich ähnlichen Polypeptiden erkannt. *Microcystis* FDF has a LD_{50} (IP, Mäuse) von 0.5 mg/kg; durch Gefriertrocknung erhaltene *Microcystis* - Zellen haben LD_{100} (*per os*, verschiedene Tiere) von 1 bis 2 Gramm per Kilogramm. *Anabaena* VFDF ist vielleicht ein Exotoxin. In seinen Eigenschaften gleicht es dem *Microcystis* VFDF.

Die Entwicklung einer giftigen Wasserblüte hängt vom günstigen Zusammenwirken von mindestens sechs Veränderlichen ab : 1. Gegenwart und Vorherrschen eines giftigen Stammes von Algen, 2. Grösse und Zusammensetzung der die Algen begleitenden Bakterienflora, 3. Be-sufficient amounts of toxin at the right time by susceptible species of animals.

The effects of toxic waterblooms on various other organisms are discussed.

d'une culture toxique d'algue, (2) le volume et la composition de la flore bactérienne associée, (3) les conditions requises pour la production de toxines par les algues, (4) les conditions requises pour ressembler de larges masses d'algues près du rivage, (5) les conditions requises pour libérer des toxines sans trop les diluer ou les détruire, (6) l'ingestion de quantités suffisantes de toxine, au bon moment, par certains animaux susceptibles.

On discute aussi des effets des floraisons toxiques sur d'autres organismes variés.

dingungen nötig für Toxinerzeugung durch die Algen, 4. Günstige Bedingungen für die Ansammlung grosser Algenmassen nahe der Küste, 5. Bedingungen nötig zur Abgabe des Toxins ohne zu viel Verdünnung oder Zerstörung und 6. Rechtzeitige Einnahme genügender Toxinmengen durch eine dafür empfindliche Tiergattung.

Die Wirkungen giftiger Wasserblüten auf verschiedene andere Organismen werden besprochen.

SOME ENVIRONMENTAL EFFECTS OF COAL AND GOLD MINING ON THE AQUATIC BIOTA. A. D. Harrison. p. 270. The effects of coal and gold mining are discussed in the light of experience in South Africa. There are two main effects on streams from both types of mining: silting and pollution by acid sulphates. The latter is the more serious. Acid sulphates, resulting from bacterial action or pyrite rocks and ores, contaminate effluents from mines and mine dumps, which frequently have a pH as low as 2.3 and contain dissolved sulphates of the order of 2000 ppm or more. In polluted streams and swamps when the pH is constantly below 5, characteristic associations of plants and animals appear. These are discussed. After neutralization, high sulphate values of up to 1000 ppm appear to have little effect on the normal biota. Where pollution is periodic and pH values fluctuate from very acid to alkaline, the biota is greatly impoverished and characteristic associations are not found.

QUELQUES CONDITIONS DU MILIEU DÉTERMINÉES PAR LES MINES DE CHARBON ET D'OR ET LEUR EFFET SUR LA VIE AQUATIQUE. A. D. Harrison. p. 270. On a étudié ici les effets de l'exploitation des mines de charbon et d'or en se servant d'expériences en Afrique du Sud. Ces exploitations minières produisent deux effets principaux sur les cours d'eau, ce sont l'envasement et la pollution par les sulfates acides. Le dernier effet est plus sérieux. Les sulfates acides, provenant de l'action des bactéries sur les roches pyriteuses et sur le minerai contaminent les rivières près des mines et près des dépôts de minerai. Ces rivières ont parfois un pH très bas, de l'ordre de 2.3 et contiennent des sulfates dissous dans l'ordre de 2,000 ppm ou plus. Dans les rivières et les marais pollués, lorsque le pH se maintient constamment en bas de 5, il se forme des associations caractéristiques de plantes et d'animaux qu'on discute ici. Après la neutralisation, les concentrations élevées en sulfate, jusqu'à 1,000 ppm semblent avoir bien peu d'effet sur le contenu biologique normal. Quand la pollution est périodique et que le pH subit des fluctuations indiquant alternativement un milieu très acide et alcalin, le contenu biologique est fortement appauvri et on ne trouve pas d'associations caractéristiques.

EINIGE UMGEBUNGSEINFLÜSSE VON KOHLEN - UND GOLDBERGARBEIT AUF DAS LEBEN IM WASSER. Arthur D. Harrison. P. p. 270. Im Lichte von Erfahrungen in Südafrika werden die Einflüsse von Kohlen - und Goldbergarbeit besprochen. In beiden Fällen sind Verschlickung und Verschmutzung durch saure Sulfate die Haupteinflüsse und jener durch Sulfate muss ernster genommen werden. Saure Sulfate entstehen durch Bakterienwirkung auf Schwefelkiesgesteine und - erze; sie verunreinigen die Abwässer von Bergwerken und Halden, welche dann oft eine pH Stufe von 2, 3 haben und gelöste Sulfate in der Grössenordnung von 2 g/1 (= 2 000 parts per million) oder noch mehr enthalten. In verunreinigten Strömen und Sümpfen treten, wenn die pH Stufe dauernd unter 5,0 liegt, charakteristische Verbände von Tieren und Pflanzen auf, welche besprochen werden. Nach Neutralisierung scheinen hohe Sulfatwerte bis zu 1 g/1 (= 1 000 parts per million) wenig Wirkung auf die normale Lebewelt zu haben. Wo die Verunreinigungen in regelmässigen Zeitabständen auftreten und die pH Werte sich zwischen stark sauer und stark alkalisch bewegen, da verarmen die Gegenden sehr an Lebewesen und die charakteristischen Verbände werden nicht mehr gefunden.

CONTROL OF FISH PARASITES. Glenn L. Hoffman. p. 283. Fish infected with fungi, protozoa, helminths, and parasitic copepods may be unsuitable for experimental use, including the bioassay of polluted water. Methods of prophylaxis, and treatment if known, are discussed.

Saprolegnia and related fungi can be controlled with malachite green.

CONTROLE DES PARASITES DU POISSON. Glenn L. Hoffman. p. 283. Le poisson infecté par fungi, protozoa, helminths et copepods parasitiques n'est pas apte à l'utilité expérimentale, incluant les essais d'eau polluée. Les méthodes de prophylaxie et de traitement connu, sont décrites. *Saprolegnia* et fungi peuvent être controlés par malachite verte. La plupart de protozoa

KONTROLLE VON FISCHPARASITEN. Glenn L. Hoffman. p. 283. Fische, welche mit Pilzen, Urtierchen, Würmern und parasitischen Krustentieren infiziert sind, können für Versuchszwecke, einschliesslich von Bioversuchen an verunreinigtem Wasser, ungeeignet sein. Vorbeugungsmethoden und, wenn bekannt, Behandlungsverfahren werden besprochen.

Most external protozoa can be removed with formalin, potassium permanganate, etc. *Hexamita*, an intestinal parasite, can be eradicated with carbarsone, calomel, etc.

Intestinal helminths can be removed with di-n-butyl-tin oxide, but there is no known chemotherapy for fish tissue parasites. Leeches and parasitic copepods can sometimes be removed with insecticides, and predacious beetle larvae can be killed with kerosene on the pond.

Prophylaxis is probably more important than treatment. This involves selection of parasite-free stock, control of intermediate hosts, and external prophylactic treatment of fish at time of collection or transfer.

externes peuvent être extrait par formaline, potassium permanganate etc. *L'Hexamita*, un parasite intestinal peut être déraciné par carbarsone, calomel, etc.

Les helminths intestinales peuvent être extraites avec di-n-butyl-oxide d'étain mais rien n'est connu pour les parasites des tissues de poisson. Les sangsues et les copépodes parasitiques peuvent parfois être extraits par des insecticides et les larves de coleoptère peuvent être tuées par le kérosène sur les étangs. La prophylaxie est probablement plus importante que le traitement. Ceci implique une sélection de parasites libres, un contrôle de traitement prophylatique externe du poisson au moment du ressemblement ou du transfert.

Saprolegnia und verwandte Pilze können mit Malachitgrün im Zaume gehalten werden. Die meisten Urtierchen an Oberflächen können mit Formalin, Kaliumpermanganat usw. entfernt werden. *Hexamita*, ein Eingeweideparasit, ist mit Carbarsone, Kalomel usw. ausrottbar.

Eingeweidewürmer können mit Di-n-butylzinnoxyd entfernt werden, gegen Fischgewebeparasiten ist jedoch bisher keine chemotherapeutische Methode bekannt. Blutegel und parasitische Krustentiere sind manchmal mit Insektenvertilgungsmitteln entfernbar und Käferlarven können durch eine Schicht von Kerosin auf der Oberfläche von Teichen getötet werden.

Vorbeugung ist wahrscheinlich wichtiger als Behandlungsmethoden. Das bedeutet Wahl von parasitenfreiem Fischbesatz, Kontrolle von Zwischenwirten und vorbeugende Oberflächenbehandlung der Fische beim Sammeln und beim Überführen von einer Stelle zur andern.

HOW SHOULD AGRICULTURAL POLLUTANTS BE CONTROLLED. C. H. Hoffmann. p. 253. There is no question as to the importance and necessity of agricultural chemicals in the production and conservation of food, feed, and fiber, and in protecting plants, animals, and man from serious diseases. With the increased use of these chemicals, however, there is great concern about their persistence in the soil, particularly as potential water pollutants. Recent studies regarding agricultural chemicals as water pollutants are reviewed in order to indicate the magnitude of the problem. There are several approaches to the control of agricultural pollutants, including the enactment of federal and state laws and regulations permitting the use of agricultural chemicals under specified conditions; supervised pest control programs in which trained and responsible operators use toxic materials; and the establishment of federal or state pest control review boards, representing different agencies and interests to assure that widespread control programs are conducted in the public interest. The importance of educational activities and good public relations cannot be overemphasized in connection with the general safe use of agricultural chemicals to avoid unnecessary pollution. The interest of various national societies and other groups representing different disciplines concerned with various

CONTRÔLE DES PRODUITS AGRICOLES POLLUANTS. C. H. Hoffmann. p. 253. Il n'est pas question de revenir sur l'importance et la nécessité des produits chimiques agricoles dans la production et la conservation des aliments et des fourrages et dans la protection des plantes, des animaux et même de l'homme contre des maladies graves. Cependant, avec l'emploi toujours plus grand de ces produits, il existe un grave problème au sujet de leur persistance dans le sol, particulièrement vis à vis d'une pollution possible des eaux. Des études récentes concernant les produits chimiques agricoles en tant que substances polluantes sont passées en revue afin de montrer l'ampleur du problème. Il existe plusieurs moyens d'aborder le contrôle de substances polluantes comprenant: la promulgation de lois fédérales et d'état et des réglementations autorisant l'emploi des produits chimiques agricoles dans des conditions bien déterminées; des programmes de destruction dirigées où des opérateurs instruits et responsables emploient les produits toxiques; et l'établissement de conseils fédéraux au d'état pour la destruction des nuisibles groupant les réprésentants des differents intérêts pour s'assurer que les campagnes de pulvérisation sont menées en vue de l'intérêt public. L'importance des activités d'enseignement et de bonnes vulgerisations ne peut être trop soulignée

WIE SOLLTEN LANDWIRTSCHAFTLICHE WASSERVERUNREINIGUNGEN ÜBERWACHT WERDEN? Clarence H. Hoffman. p. 253. Die Wichtigkeit und Notwendigkeit von landwirtschaftlichen Chemikalien in der Erzeugung und Erhaltung von Nahrungs - und Futtermitteln und von Faserstoffen und zum Schutz von Pflanzen, Tieren und Menschen gegen ernste Erkrankungen steht ausser Zweifel. Mit zunehmender Anwendung dieser Chemikalien wächst indessen die Besorgnis über ihre Beharrung im Boden, besonders als mögliche Verunreinigungen des Wassers. Neuere Studien auf diesem Gebiet werden besprochen um die Grösse des Problems darzulegen. Man kann die Überwachung dieser landwirtschaftlichen Verunreinigungen auf verschiedenen Wegen angehen, z. B. durch Bundes - und Staatsregelungen und Gesetze, welche die Anwendung landwirtschaftlicher Chemikalien unter bestimmten Bedingungen gestatten, durch beaufsichtigte Ungezieferkontrollmassnahmen, in welchen geschultes und verantwortliches Personal diese giftigen Stoffe anwendet und durch Errichtung von Bundes - oder Staatsausschüssen zur Überwachung der Ungezieferkontrolle, in welchen die verschiedenen Behörden und Interessenten vertreten sind, damit Gewähr dafür gegeben ist, dass die umfassenden Kontrollmassnahmen im öffentlichen Interesse liegen. Die Wichtigkeit erzieherischer Tätigkeit und guter Beziehungen zur

aspects of water pollution which hold seminars, symposia, and conferences to consider various problems is not only stimulating to research and control workers but also the publicity associated with such meetings enables the public to become better informed. Moreover, some organizations help finance studies on special problems or seek support for research needed to answer urgent problems. The role of research in the discovery of new pest control materials that are less toxic to other organisms and, therefore, of less consequence as water pollutants is emphasized. In addition, more research is needed on nonchemical methods of pest control, which would obviate some water pollution problems.

en liaison avec un emploi rationnel des produits chimiques agricoles pour éviter une pollution superflue. Il est intéressant que différentes sociétés nationales et des groupes représentant des disciplines différentes touchant les divers aspects de la pollution des eaux, tiennent des séminaires, symposia ou conférences pour étudier ces problèmes car non seulement ils stimulent la recherche et ceux qui contrôlent les opérations, mais la publicité faite autour de ces réunions permet de mieux informer le public. De plus quelques organisations aident financièrement des études sur des problèmes particuliers ou cherchent une aide pour résoudre des problèms urgents. Le rôle de la recherche dans la découverte de nouveaux pesticides moins dangereux pour les autres organismes, donc moins susceptibles de polluer, est soulignée. De plus, il est nécessaire de développer les recherches sur les méthodes de contrôle non chimiques, ce qui pourrait prévenir d'autres problèmes de pollution.

Öffentlichkeit kann im Zusammenhang mit der allgemeinen sicheren Anwendung landwirtschaftlicher Chemikalien zur Vermeidung unnötiger Verunreinigung nicht genug betont werden. Verschiedene nationale Gesellschaften und Interessengruppen sind durch die Vielseitigkeit des Problems der Wasserverunreinigung beunruhigt und halten Seminare, Symposien und Konferenzen zur Besprechung der auftretenden Fragen; solche Tätigkeit ist einerseits anregend für Forscher und Kontrollpersonal, andererseits aber auch von ganz allgemeiner Wichtigkeit, weil die Werbekraft solcher Zusammenkunfte der Öffentlichkeit eine bessere Sachkenntnis vermittelt. Ausserdem unterhalten einige Vereinigungen oder Körperschaften das Studium von Sonderfragen durch Geldzuwendungen oder werben für geldliche Unterstützung von Forschungsarbeiten über dringende Fragen. Die Rolle der Forschung in der Entdeckung neuer Ungeziefervertilgungsmittel von geringerer Giftigkeit gegen erwünschte Organismen und deshalb geringerer Gefährlichkeit als Wasserverunreinigungen wird hervorgehoben. Mehr Forscherarbeit an nichtchemischen Verfahren der Ungezieferkontrolle ist nötig; dadurch könnten zum mindesten einige der Wasserverunreinigungsprobleme beseitigt werden.

RELATIONS OF PLANKTONIC CRUSTACEA TO DIFFERENT ASPECTS OF POLLUTION. J. Hrbacek. p. 53 . The following are discussed: occurrence of common planktonic species of Crustacea in different saprobiological degrees; subjectivity in estimating the dependence of planktonic species of Crustacea on the extent of saprobity and efforts for development of a more objective approach; and relations of different groups of planktonic Crustacea to various primary or secondary consequences of pollution:

1. Abiotic — pH, oxygen, hydrogen sulphide, and toxic substances.
2. Biotic — the concentration of food particles; the influence of predation.

There are relatively large differences between morphologically closely-related species of the genus *Daphnia* in the ability to survive under different nutritional conditions. In ponds with good fishstock, observations illustrate that the abundance of the predominant species of planktonic Crustacea is reduced. A new interpretation of the possible causes of changes in species com-

RELATIONS ENTRE LES CRUSTACÉS PLANCTONIQUES ET DIFFERENTS ASPECTS DE LA POLLUTION. J. Hrbacek. p. 53 . Les sujets suivants sont discutés: présence d'espèces communes de crustacés planctoniques dans les différentes zones de saprobies; subjectivité dans l'estimation de la dépendance des espèces de crustacés planctoniques vis à vis de l'évaluation des saprobies, et efforts pour développer des méthodes plus objectives; relations de différents groupes de crustacés planctoniques avec diverses conséquences primaires ou secondaires de pollution:

1. abiotique - pH oxygène, hydrogène sulfuré et substances toxiques
2. biotique - concentration des particules nutritives, influence des prédateurs.

Il y a relativement beaucoup de différence entre des espèces très proches morphologiquement du genre *Daphnia* en ci qui concerne leur possibilité de survie dans des conditions de nutrition différente. Dans des étangs riches en poissons, des observations montrent que l'abondance des espèces dominantes est réduite. L'auteur donne une

DIE BEZIEHUNGEN ZWISCHEN PLANKTONKRUSTAZEEN UND VERSCHIEDENEN VERUNREINIGUNGSFRAGEN. Jaroslav Hrbacek. p. 53 . Folgende Probleme werden besprochen: das Vorkommen gewöhnlicher Planktonarten von Krustazeen in verschiedenen saprobiologischen Graden; die persönliche Gleichung in der Beurteilung der Abhängigkeit der Planktonarten der Krustazeen vom Saprobiensystem und die Bemühungen um die Entwicklung einer sachlichen Bewertung; und schliesslich die Beziehungen der verschiedenen Gruppen von Planktonkrustazeen zu weiteren hauptsächlichen oder untergeordneten Folgen der Verunreinigung, nämlich 1. abiotisch - pH, Sauerstoff, Schwefelwasserstoff und Giftstoffe 2. biotisch - Nahrungskonzentration, Einfluss von Predation

Morphologisch nahe verwandte Arten der Gattung *Daphnia* unterscheiden sich beträchtlich in ihrer Fähigkeit verschiedene Ernährungsbedingungen zu überleben. In Weihern mit gutem Fischbesatz ist die Häufigkeit der vorherrschenden Art von Planktonkrustazeen herabgesetzt. Eine neue Erklärung der position of zooplankton in polluted lakes and reservoirs is given.

A more complete knowledge of physiological differences of species is needed for understanding ecological effects of pollution on different species of planktonic Crustacea and for utilizing these species as biological indicators for water purity control purposes.

nouvelle interprétation des causes possibles de changement dans la composition des espèces de zooplancton dans les lacs et réservoirs pollués.

Une connaissance plus complète des différences physiologiques des espèces est nécessaire pour comprendre les influences écologiques des pollutions sur les crustacés planctoniques et pour utiliser ces espèces comme indicateurs biologiques dans les controles de qualité des eaux.

möglichen Ursachen der Änderung in der Artenzusammensetzung des Zooplankton in verunreinigten Seen und Stauseen wird gegeben.

Mehr Kenntnis der physiologischen Unterschiede der Arten ist zum Verständnis der ökologischen Wirkungen von Verunreinigung auf verschiedene Arten von Planktonkrustazeen und zur Anwendung dieser Arten als biologische Anzeiger für Wasserreinheit nötig.

WATER QUALITY CRITERIA FOR FISH LIFE. Marcel Huet. p. 160. For a particular habitat to be suitable for fish, it is necessary that the fish should be able to live and develop there. This means that the environmental factors for respiration, feeding, and reproduction should be suitable. Environmental requirements vary considerably from one kind of fish to another. Within various types of water, the following distinctions should be made: salmonid waters, mixed waters, and cyprinid waters.

Reasonably precise criteria can only be given for certain factors: temperature, dissolved oxygen, and pH. Care must be taken to avoid confusing the extremes for these factors and the optimum value for the same factor. These details are discussed in the text. Other environmental factors are also discussed.

CRITÈRES DE QUALITÉ DE L'EAU EN RAPPORT AVEC LA DURÉE DE VIE DU POISSON. Marcel Huet. p. 160. Pour qu'un habitat particulier soit convenable pour le poisson, il est nécessaire que le poisson puisse se développer et vivre dans ce milieu. Ceci implique que les facteurs environnants pour la respiration, l'alimentation et la reproduction soit convenables. Les conditions requises varient considerablement d'une sorte à une autre. Entre les types variés d'eau, les distinctions suivantes doivent être faites: eaux «salmonides» eaux mélangées, et eaux cyprinides.

Les critères précis peuvent seulement être donnés raisonnablement pour certains facteurs: température, oxygène dissous, et pH. Des précautions doivent être prises pour éviter la confusion des extrêmes de ces facteurs et la valeur optimum pour le même facteur. Ces détails sont discutés dans le texte. D'autres facteurs environnants sont aussi discutés.

KENNZEICHEN DER WASSERGÜTE GEGENÜBER FISCHEN. Marcel Huet. p. 160. Die Eigenschaften der Umgebung, welche für das Leben und Gedeihen von Fischen, für deren Atmung, Stoffwechsel und Fortpflanzung notwendig sind, wechseln mit der Gattung der Fische. Innerhalb verschiedener Wassertypen sollten die folgenden Unterscheidungen gemacht werden: salmonide, gemischte und cyprinide Wasser.

Bestimmte Kennziffern können nur für einige Eigenschaften wie Temperatur, gelösten Sauerstoff und pH gegeben werden. Höchst und Mindestwerte für diese Faktoren dürfen nicht mit dem Bestwert verwechselt werden. Diese Einzelheiten und andere Eigenschaften der Umgebung werden im Text besprochen.

BACTERIA-PROTOZOA INTERACTIONS IN PURIFYING WATER. S. H. Hutner. p. 45. Unless insecticides, plant growth regulators, detergents, and other products of the synthetic organic chemicals industry can be microbially degraded, they may accumulate in soils and contaminate water supplies. To the author's knowledge a realistic laboratory test to predict the susceptibility of synthetics is not available. The problem can be put operationally as: What is the smallest, quickest developing laboratory microcosm that reflects the macrocosm represented by, say, a sewage lagoon? Recent findings that certain photosynthetic particle-ingesting flagellates are ubiquitous in fresh waters, soils, and the ocean, emphasize that conventional dark enrichments are inadequate; enrich-

INTERACTION DES BACTÉRIE-PROTOZOAIRES. S. H. Hutner. p. 45. Excepté que les insecticides, les auxines, les détergents, et les autres produits organiques de l'industrie chimique peuvent être dégradés au niveau du microbe, ils peuvent s'accumuler dans le sol et contaminer l'alimentation d'eau. A la connaissance de l'aûteur, un bon test de laboratoire pour prédire la suceptibilité relativement aux synthétique n'est pas disponible.

Le problème peut être mis sous la forme: «quel est le plus petit, le plus rapide dévelopement microcosme en laboratoire qui reflète le macrocosme représenté par un lagon d'eau stagnante? Des recentes recherches ont signalées que certaines flagellaires photosynthesantes sont présentes dans les eaux douces,

EINWIRKUNG VON BAKTERIEN UND PROTOZOEN AUFEINANDER IN DER WASSERREINIGUNG. S. H. Hutner. p. 45. Schädlingsvertilgungsmittel, Pflanzenwuchshormone, oberflächenaktive Waschmittel und andere Erzeugnisse der synthetischen organischen Chemie können sich, wenn sie nicht mikrobiologisch abbaubar sind, in Böden anreichern und die Wasserversorgung verunreinigen. Soweit dem Verfasser bekannt ist, gibt es bisher keine zuverlässige Laboratoriumsprobe, welche die Empfindlichkeit gegenüber solchen synthetischen Stoffen vorauszusagen gestattet. Praktisch kann man die Frage so stellen: welcher kleinste raschest wachsende Laboratoriumsmikrokosmos würde die Verhältnisse in einem Makrokosmos, etwa in einer Abwasserlagune, wirklich-

ment cultures with test substrates might well be conducted under mixed incandescent lamps (to encourage photosynthetic bacteria) and fluorescent lamps. A related problem is the degradability of the carcinogenic hydrocarbons; this in turn is bound up with the question of how the body detoxifies unnatural compounds and which protozoa are the best indices of toxicity to higher animals.

THE SIGNIFICANCE OF MACRO-INVERTEBRATES IN THE STUDY OF MILD RIVER POLLUTION. H. B. N. Hynes. p. 235. As biological indicators of pollution, at least initially, small organisms are easier and more satisfactory to work with than fish. A micro-invertebrate analysis tells one if the situation is worth investigating further. Since such a survey is based on no hard and fast system, it is flexible and allows the investigator to look afresh at each problem and to interpret the data in the light of prevailing circumstances. If the survey reveals little change in the invertebrate fauna, it is reasonable to assume that the fish are not affected and that the water is suitable for industrial use.

The examples given of such surveys show that a very simple study of invertebrates can be used to determine how far the effects of pollution extend, even when it is no longer occurring, and also to check on recovery when the pollution is being abated.

sols et les océans et démontrent que les enrichements conventionnel au noir ne sont pas adequates. La culture d'enrichment avec test de réaction peut bien être conduit sous lampe incandescente (enfin d'encourager la bactérie photosynthetique) et flourescente mélangées. Un problème relaté est la dégradation de l'hydrocarbure cancérigene; ceci est en relation avec la manière ou le corps détoxifie les composants non naturel et que les protozoaires sont le meilleur indice de toxiquité aux animaux d'especes plus complètes.

L'IMPORTANCE DES INVERTÉBRÉS DE GRANDE TAILLE DANS L'ÉTUDE DE LA POLLUTION LÉGÈRE DES RIVIÈRES. H. B. N. Hynes. p. 235. Il est plus satisfaisant et plus facile de se servir, du moins pour commencer, de petits organismes plutôt que de poissons, comme indicateurs de pollution. Une analyse des invertébrés de grande taille indique s'il est nécessaire de continuer les travaux. Comme un relevé de ce genre n'est pas basé sur une méthode rigoureuse et fixe, mais flexible, ceci permet au chercheur d'examiner de nouveau chaque problème et d'interpréter les résultats à la lumière des circonstances actuelles. Si on ne trouve que peu de changements dans la faune des invertébrés, il est raisonnable de croire que les poissons ne sont pas affectés et que l'eau peut être utilisée par l'industrie.

Les exemples des relevés de ce genre indiquent qu'une très simple étude des invertébrés peut être utilisée pour déterminer jusqu'où les effets de la pollution se font sentir, même quand elle a cessé d'exister et aussi pour vérifier l'épuration quand la pollution est en train de diminuer.

keitsnah wiedergeben? Neuere Entdeckungen über die weite Verbreitung gewisser photosynthetischer Geisseltierchen, welche feste Teilchen als Nahrung aufnehmen, in Gewässern, Böden und im Meere betonen, dass die üblichen Anreicherungen im Dunklen unzureichend sind; Anreicherungskulturen mit Versuchssubstraten können sehr wohl unter Mischlicht (Glühlampen zur Förderung des Wachstums photosynthetischer Bakterien und Leuchtröhren) ausgeführt werden. Ein verwandtes Problem ist die Abbaumöglichkeit krebsverursachender Kohlenwasserstoffe; diese Frage wiederum ist mit der anderen, wie der Körper Fremdstoffe entgiftet und welche Protozoen sich am besten als Anzeiger von Giftigkeit für höhere Tiere eignen, eng verbunden.

DIE BEDEUTUNG DER MAKRO-WIRBELLOSEN FÜR DAS STUDIUM GERINGER FLUSSVERUNREINIGUNGEN. H. B. N. Hynes. p. 235. Als biologische Anzeiger von Verunreinigungen verwendt man, wenigstens in Vorversuchen, mit Vorteil kleine Organismen anstatt Fische. Eine Analyse der Makro-Wirbellosen lässt erkennen, ob ein Fund ein eingehendes Studium wert ist. Da eine solche Untersuchung nicht auf ein starres System aufgebaut ist, so erlaubt sie dem Forscher, unbeeinflusst jede neue Fragestellung anzugehen und sie im Lichte der gerade herrschenden Umstände zu erklären. Wenn die Überprüfung wenig Änderung in der Wirbellosenfauna zeigt, dann kann man annehmen, dass Fische nicht beeinflusst werden und dass das Wasser für industrielle Zwecke geeignet ist.

Beispiele fur solche Überprüfungen werden gegeben, welche zeigen, dass man durch ein sehr einfaches Studium der Wirbellosen feststellen kann, wie weit sich die Wirkungen der Verunreinigung erstrecken, sogar wenn die Verunreinigung selbst gar nicht mehr besteht; auch kann man nach Behebung der Verunreinigung den jeweils erreichten Reinheitsgrad bestimmen.

THE CONTRIBUTION OF BOTTOM MUDS TO THE DEPLETION OF OXYGEN IN RIVERS AND SUGGESTED STANDARDS FOR SUSPENDED SOLIDS. Peter C. G. Isaac. p. 346 . Questions are posed as to whether bottom muds in sedimented or resuspended states contribute to the oxygen demand of rivers, and whether it is feasible to impose suspended-solids standards on effluents to limit bottom deposits. The influence on oxygen demand of dissimilation products from the sediment is discussed.

It has been well-established that bottom and resuspended muds do exert an oxygen demand. The relative contributions of settled deposits and resuspended solids to oxygen demand vary widely according to the degree of resuspension. Resuspended bottom muds cause a marked reduction in the dissolved oxygen concentration.

Several investigators have concluded that the oxygen demand of sludge deposits is independent of the dissolved oxygen concentration in the supernatant water. It is the rate of upward diffusion to the surface of the sediment of the oxidizable substances produced anaerobically within the deposits that controls the rate of benthal oxygen demand.

The extent to which bottom muds are resuspended in flowing water varies greatly according to circumstances prevailing in different rivers. The amount of resuspension determines the degree of oxygen depletion, which is much greater from suspended solids than from stationary bottom deposits. Laboratory tests showed that a 1-ppm concentration of detergent (ABS) decreased the settling rate of any given concentration of mud.

Control of the amount of suspended solids discharged to a stream or lake is definitely required. Such control will normally result in a relatively non-turbid stream. The quality of the diluting water must be specified since water taking up much dissolved oxygen is, in itself, of doubtful purity.

CONTRIBUTION DES VASES DU FOND À LA DIMINUTION DE L'OXYGÈNE DANS LES RIVIÈRES ET SUGGESTIONS POUR LE CONTRÔLE DES SOLIDES EN SUSPENSION. Peter C. G. Isaac. p.346. On se demande si les vases du fond en dépôt au en suspension pourraient être en partie responsable des quantites d'oxygène utilisées par les rivières et s'il y aurait moyen d'imposer des étalons au sujet des solides en suspension dans les effluents afin de limiter les dépôts du fond. On discute de l'influence des produits de dégradation des sédiments sur la demande d'oxygène.

Il a été établi définitivement que les vases sur le fond et de nouveau en suspension utilisent une certaine quantité d'oxygène. Les dépôts en sédiments et les solides de nouveau en suspension ont des effets bien variés sur l'oxygène dissous, ces effets varient selon le degré de suspension renouvelée. Les vases du fond en suspension causent une réduction très marquée sur la concentration de l'oxygène dissous.

Plusieurs chercheurs en sont venus à la conclusion que la demande d'oxygène des dépôts de vases ne dépendrait pas de la concentration de l'oxygène dissous dans la couche d'eau immédiatement superposée. Il semble que l'utilisation benthique de l'oxygène a un taux contrôlé par la diffusion verticle vers la surface de la couche sédimentaire, diffusion des substances oxydables produites anaérobiquement au sein même des dépôts.

Dans l'eau courante, la suspension de la vase du fond varie beaucoup suivant les conditions qui existent dans les differentes rivières. La quantité des solides de nouveau en suspension détermine le degré de perte d'oxygène qui est beaucoup plus grand lorsque les solides sont en suspension que lorsqu'ils sont déposés au fond. Des essais de laboratoire ont montré que l'addition d'un détersif de l'ordre de 1-ppm diminue le taux de sédimentation de la vase quelle qu'en soit la concentration.

Il faudrait certainement établir un contrôle sur les solides en suspension déversés dans les cours d'eau et les lacs. Un contrôle de ce genre devrait normalement avoir pour résultant un cours d'eau relativement clair. Il faudrait aussi tenir compte de la qualité de l'eau diluante (affluent), car l'eau qui utilise une grande quantité de l'oxygène dissous est sûrement loin d'être pure.

DER BEITRAG DER BODENSCHLÄMME ZUR ERSCHÖPFUNG DES SAUERSTOFFS IN FLÜSSEN UND VORSCHLÄGE VON NORMEN FÜR SCHWEBESTOFFE. Peter Charles Gerald Isaac. p. 346 . Die Fragen werden aufgeworfen, ob Bodenschlämme in abgesetztem oder in wieder suspendiertem Zustand zum Sauerstoffbedarf von Flüssen beitragen und ob es möglich ist, die Normen für Schwebstoffe auf Abflüsse zur Abgrenzung der Bodenablagerungen zu übertragen. Der Einfluss von Abbaustoffen des Bodenschlamms auf den Sauerstoffbedarf wird besprochen.

Es ist wohl bekannt, dass Bodenschlamm und wieder suspendierter Schlamm den Sauerstoffbedarf vergrössern. Die verhältnismässigen Beiträge von zu Boden gesunkenen Ablagerungen und von wieder suspendierten Feststoffen zum Sauerstoffbedarf wechseln sehr in Abhängigkeit vom Grad der Wiedersupendierung. Wieder suspendierte Bodenschlämme verursachen eine bedeutende Verminderung der Konzentration des gelösten Sauerstoffs.

Verschiedene Forscher sind zu dem Schluss gekommen, dass der Sauerstoffbedarf von Schlammablagerungen von der Konzentration des im darüberstehenden Wasser gelosten Sauerstoffs unabhängig ist. Das Verhältnis von Diffusion nach oben zur Oberfläche des Bodensatzes der anaerob im Bodensatz erzeugten oxidierbaren Stoffe steuert die Grösse des Sauerstoffbedarfs der auf dem Boden lebenden Fauna.

Der Grad der Wiedersuspendierung von Bodenschlamm in fliessendem Wasser ist je nach den in den Flüssen herrschenden Bedingungen sehr verschieden. Der Betrag dieser Wiedersuspendierung bestimmt den Grad der Sauerstoffverarmung, welcher viel grösser ist, wenn nicht der unveränderliche Bodensatz, sondern Schwebestoffe seine Ursache sind. Laboratoriumsversuche zeigten, dass eine Konzentration von 1mg/1 eines oberflächenaktiven Waschmittels (ABS) die Senkgeschwindligkeit jeder beliebigen Konzentration von Schlamm verringerte.

Überwachung der Menge von Schwebestoffen, die in einen Fluss oder See entleert werden, ist bestimmt nötig. Diese Überwachung wird gewöhnlich zu einem verhältnismässig klaren Flusswasser führen. Die Güte des Verdünnungswassers muss angegeben werden, da ein Wasser, das zu viel gelösten Sauerstoff aufnimmt, schon an und für sich von zweifelhafter Reinheit ist.

THE USE OF CHARCOAL FOR COLLECTING INSECTICIDES IN WATER SAMPLES. Charles C. Van Valin and Burton J. Kallman. p. 250 . To replace the time-consuming and often unreliable method of solvent extraction of water samples, a method has been devised that utilizes small quantities of charcoal for the adsorption of pesticides from relatively small quantities of water. In practice, 1-gallon samples of water are shaken with 10 grams of charcoal. The charcoal is filtered, briefly air-dried, and sent to the laboratory for extraction and subsequent analysis. With heptachlor and heptachlor epoxide, recoveries of 60 to 90 percent, respectively, have been achieved in the 25 to 500 microgram range, with a paper chromatographic analytical procedure. Recoveries for other pesticides remain to be determined.

L'UTILITÉ DU CHARBON DE BOIS EN VUE DE COLLECTER LES INSECTICIDES DANS DES ÉCHANTILLON D'EAU. Charles C. Van Valin and Burton J. Kallman. p. 250 . Pour remplacer un essai à long terme et souvent une méthode peu sure d'extraction de dissolvant d'échantillon d'eau, une methode a été essayée, utilissant de petites quantités de charbon de bois, vu la quantité relativement petite de l'échantillon d'eau, pour l'absorption de pesticides. En pratique, 1-gallon d'eau est agitée avec 10 grammes de charbon de bois. Le charbon de bois est filtré, légèrement seché à l'air et envoyé au laboratoire pour extraction et analyse complémentaire. Avec heptachlore et heptachlore epoxide, recouvrement respectivement à 60 et 90% ont été obtenus dans une domaine de 25 a 500 microgrammes avec un procédé d'analyse utilisant un papier chromatagraphique. L'etablissement pour d'autres pesticides reste encore à déterminer.

DIE ANWENDUNG VON HOLZKOHLE ZUM ENTFERNEN VON SCHÄDLINGSVERTILGUNGSMITTELN AUS WASSERPROBEN. Charles C. Van Valin and Burton J. Kallman. p. 250. Als Ersatz für die zeitraubende und oft unzuverlässige Methode der Extraktion von Wasserproben mit Lösungsmitteln wurde ein Verfahren ausgearbeitet, welches kleine Mengen Holzkohle für die Adsorption von Schädlingsvertilgungsmitteln aus relativ geringen Wassermengen benützt. In der praktischen Anwendung wird eine Gallone (USA, etwa 3,79 l) der Wasserprobe mit 10 Gramm Holzkohle geschüttelt. Die Holzkohle wird abgefiltert, kurz an der Luft getrocknet und zwecks Extraktion und darauffolgender Analyse ins Laboratorium geschickt. Mit Heptachlor (1,4,5, 6,7,7,8-heptachlor-3α, 4, 7, 7 α-tetrahydro-4, 7-methanoinden) und Heptachlorepoxyd (1, 4, 5, 6, 7,8, 8-heptachlor-2, 3-epoxy-3α, 4,7,7α -tetrahydro-4,7-methanoinden) konnten durch Papierchromatographie im Bereich von 25 bis 500 Mikrogramm 60 bezw. 90% zurückgewonnen werden. Für andere Schädlingsvertilgungsmittel müssen die entsprechenden Werte erst noch bestimmt werden.

THE VALUE AND USE OF WATER QUALITY CRITERIA IN CONSERVATION PROGRAMS. Thomas L. Kimbell. p. 12 . Statistics are presented to illustrate the increasing water demands in this country. Conservationists are most anxious to retain our watersheds in a condition that will insure soil stability and a sustained maximum yield of quality water. This goal can be attained only through the wise use and management of timber, forage, and mineral resources. Pollution abatement offers the most difficult challenge and a real opportunity to provide the greatest single contribution toward increased usable water supply.

While state and federal water pollution controls have been strengthened, no law is effective without adequate enforcement. No water-law enforcement can be adequate without a definite set of standards established by competent research. A creditable job has been done in establishing standards for water quality as it affects human health. Very little information is available, however, on the effects of various pollutants upon fish, wildlife, and recreation potential. The number of technically trained people

LA VALEUR ET L'UTILITÉ DU CRITÈRE DE QUALITÉ DE L'EAU DANS LES PLANS DE CONSERVATION. Thomas L. Kimball. p. 12 . Les statistiques sont présentées pour illustrer l'accroissement des demandes d'eau dans ce pays. Les conservateurs sont la plupart anxieux pour garder nos lignes de partage d'eau dans une condition qui assurera une stabilité du sol et soutiendra un rendement maximum de qualité d'eau. Ce but peut être atteint seulement par la manière utilisée et la gestion des bois de construction, du forage et des ressources minérales. La diminution de la pollution offre la sommation la plus difficile, et une réele occasion de fournir la plus grande contribution du coté de l'accroissement d'alimentation en eau potable.

Tandis que les contrôles d'état et fédéraux au sujet de la pollution de l'eau ont été renforcés, aucune lois n'est effective sans une application adéquate. Aucune application de lois en rapport avec l'eau ne peuvent être adéquate dans un jeu defini de programme établi par une recherche compétente. Un travail honorable a été fait en établissant des rapports de l'affection des qualités de l'eau sur la

WERT UND ANWENDUNG VON GÜTEKENNZEICHEN FÜR WASSER IN REINHALTUNGSPROGRAMMEN. Thomas L. Kimball. p. 12 . An Hand statistischer Angaben wird gezeigt, wie die Nachfrage nach Wasser in den Vereinigten Staaten ansteigt. Die mit der Reinhaltung der Gewässer beauftragten Personen sind sehr darauf bedacht, dass der Zustand der Einzugsgebiete Bodenstabilität und dauernd Höchstmengen guten Wassers gewährleistet. Dieses Ziel kann nur durch kluge Verwendung und Bewirtschaftung von Forsten, Futtermitteln und Mineralien erreicht werden. Verminderung der Wasserverunreinigung ist die schwierigste Aufgabe und sie erlaubt zweifellos, den grössten Beitrag zur Zunahme unserer Versorgung mit brauchbarem Wasser zu leisten.

Staatliche und bundesbehördliche Kontrollen der Wasserverunreinigungen wurden verstärkt, aber kein Gesetz ist ohne hinreichenden Zwang wirksam. Kein Wassergesetz ist angemessen ohne bestimmte durch Forschungsarbeiten festgelegte Normen. Solche Normen sind für Wasser für menschlichen Gebrauch vorhanden, fehlen aber fast vollkommen hinsichtlich der Wirkungen der

capable of developing water quality standards for aquatic organisms is woefully inadequate at both the state and federal level. What is needed is a sufficient research staff with laboratory facilities to develop water quality criteria for all recreational uses of water as well as for industrial and public health purposes. Standards established and backed by competent research can exercise a telling influence for good on pollution before it happens, and can have a potent and authoritative effect in the courts on those who insist on flouting the law.

santé humaine. Très peu d'information sont disponibles, cepedant il y a des informations des éffets de polluants variés sur le poisson, la vie sauvage et le potential de recréation. Le numbre de personnes techniquement entrainées capable de développer des programmes sur les qualitiés de l'eau pour les organismes aquatiques est malheurseusement non compatible au niveau fédéral et d'état. Ce qu'il faut, c'est un personnel suffisant de recherche avec des facilités au point de vue laboratoires, afin de développer le critère de qualité de l'eau, pour toutes les utilités de l'eau que ce soit dans un but industriel, ou de santé publique.

Les programmes établis et soutenus par une recherche compétente peuvent exercer une influence faste sur la pollution avant que celle-ci se déclare et peuvent avoir un effet puissant et autoritaire dans les tribunaux pour ceux qui persistent en se moquant de la loi.

verschiedenen Verunreinigungen auf Fische, Wild und Erholungsmöglichkeiten. Die Zahl der technisch Ausgebildeten, die fähig sind, Normen für die Güte des Wassers gegenüber Wassertieren und Pflanzen zu entwickeln, ist vollkommen unzulänglich in den Vereinigten Staaten wie auch in den Einzelstaaten. Henreichende Forschungskräfte mit entsprechenden Laboratorien zur Ausarbeitung von Kennzeichen der Wassergüte für alle Anwendungen von Wasser, in Industrie, zu Erholungszwecken oder zur öffentlichen Gesundheitspflege, sind nötig. Die so entwickelten Normen können dann einen grossen Einfluss sogar auf vermutete Verunreinigungen haben und können vor den Gerichten eine starke und massgebende Wirkung auf Gestzesübertreter haben.

SALINITY REQUIREMENTS OF THE FISH CYPRINODON MACULARIUS.

Otto Kinne. p. 187. Results are presented concerning the effects of salinity on the basic life processes in the euryhaline and eurythermal desert pupfish, *Cyprinodon macularius*. These results demonstrate that other environmental factors, i.e., temperature and the amount of dissolved oxygen, are capable of considerably modifying or masking the effects of salinity upon growth and food conversion in fry and maturing fish and in rates of embryonic development in eggs. Experiments were conducted under widely different salinity conditions under which this species is able to exist and reproduce in nature and in the laboratory.

A comparison between fish raised in freshwater and others raised in 35^{o}/oo salinity shows that at lower temperature levels growth is faster in freshwater, but at the higher levels growth is faster in 35^{o}/oo salinity. At 35°C food conversion is most efficient at 15^{o}/oo salinity, less efficient at 35^{o}/oo salinity, and least efficient in freshwater. Conversion efficiency also depends on temperature and other factors such as food supply and age of the fish.

Embryonic development rate is progressively retarded with increasing salinity. This retardation is significantly affected by temperature; the differences in five salinity levels are progressively augmented as the temperature in-

CONDITIONS REQUISES DE SALINITÉ DU POISSON *CYPRINODON MACULARIUS.*

Otto Kinne. p. 187. Les résultats font voir les effets de la salinité sur le processus vital d'un poisson du désert, *Cypinodon macularius*, poission euryhalin et sténotherme. Ces résultats démontrent que les autres facteurs du milieu, i.e., la température et la teneur en oxygène dissous, peuvent modifier considérablement ou masquer les effets de la salinité sur la croissance et l'alimentation des jeunes et des adultes et sur le rythme du développement embryonnaire des oeufs. On a expérimentalement soumis ce poisson à une grande variété de conditions de salinité dans lesquelles il est en mesure d'exister et de se reproduire dans son habitat et au laboratoire.

Une comparaison entre des poissons vivants, les uns en eau douce et les autres en une eau de salinité de 35^{o}/oo demontre que les poissons croissent plus rapidement à basse température en eau douce mais plus rapidement à température élevée dans une eau de salinité de 35^{o}/oo L'assimilation de la nourriture à 35°C est la plus élevée à une salinité de 15^{o}/oo moins élevée à 35^{o}/oo et la moins élevée en eau douce. Cette assimilation dépend aussi de la température et de plusieurs autres facteurs tels que la quantité de nourriture et l'âge du poisson.

Le rythe de développement embryonnaire diminue alors que la

DER SALZBEDARF DES FISCHES CYPRINODON MACULARIUS.

Otto Kinne. p. 187. Forschungsergebnisse über die Wirkung des Salzgehalts auf grundlegende Lebensvorgänge im euryhalinen und eurythermalen "desert pupfish", *Cyprinodon macularius*, werden berichtet. Sie zeigen, dass andere Umwelteigenschaften, nämlich Temperatur und Gehalt an gelöstem Sauerstoff die Wirkungen des Salzgehalts auf Wachstum und Stoffwechsel beträchtlich ändern oder verdecken können und zwar sowohl in den Jugendformen als auch in den Eiern selbst durch Änderung der Geschwindigkeit der Embryonalentwicklung. Versuche wurden innerhalb der weiten Grenzen des Salzgehaltes, zwischen welchen diese Art leben und sich fortpflanzen kann, im Freien und im Laboratorium durchgeführt.

Ein Vergleich zwischen in Süsswasser und in Wasser mit 35^{o}/oo. Salzgehalt aufgezogenen Fischen zeigt, dass das Wachstum in Süsswasser bei niedrigen, in dem Salzwasser hingegen bei hoheren Temperaturen grosser ist. Bei 35° C. ist der Stoffwechsel am besten, wenn der Salzgehalt 15^{o}/oo. beträgt; er$_{o}$ ist wenigner leistungsfähig bei 35^{o}/oo. Salzgehalt und am geringsten in Süsswasser. Seine Leistungfähigkeit hängt ausserdem noch von der Temperatur and von anderen Umständen wie Nahrungsversoguing und Alter der Fische ab.

Die Geschwindigkeit der Emcreases. The effects of salinity on embryonic development and on its upper critical and lethal temperature are highly significant statistically and easily reproducible. Nevertheless, a further analysis shows that it is not the salinity per se that causes these effects, but the concomitant change in the level of saturation for ambient oxygen. The results show that the retardation effect observed in high salinities can be nullified largely or completely by a rise in the amount of dissolved oxygen.

All the data indicate that the importance of the salinity factor as part of the total environment of *Cyprinodon macularius* can only be fully appreciated and understood within the framework of a comprehensive, poly-factorial analysis.

salinité augmente. La température affecte cette diminution sensiblement; les différences qui existent entre les cinq niveaux de salinité augmentent progressivement avec une augmentation de la température. Les effets de la salinité sur le développement embryonnaire, sur la température critique supérieure et sur la température fatale sont nettement définis au point de vue statistique et ils peuvent être répétés facilement. Cependant, une analyse poussée a démontrée que ce n'était pas la salinité *per se* qui produisait ces effets mais le changement concomitant de l'oxygène ambiant. Les résultats font voir que le retard dans le développement embryonnaire observé à salinité élevée, peut être annulé en grande partie ou totalement par une augmentation de la teneur en oxygène dissous.

Toutes les données indiquent que l'importance de la salinité comme facteur du milieu de *Cyprinodon marcularius* ne peut être évaluée et comprise que si on la considère dans une analyse poussée comme faisant partie d'une structure composée de plusieurs facteurs agissant les uns sur les autres.

bryonalentwicklung wird mit zunehmendem Salzgehalt fortschreitend verzögert. Diese Verzögerung wird durch die Temperatur erheblich beeinflusst; die Unterschiede in fünf Salzgehalten werden mit Temperaturerhöhung stufenweise grösser. Die Wirkungen des Salzgehalts auf die Embryonalentwicklung und auf ihre obere kritische und tödliche Temperatur sind statistisch sehr bedeutsam und leicht wiederholbar. Nichtsdestoweniger zeigt eine weitere Analyse, dass der Salzgehalt allein diese Wirkungen nicht verursacht, sondern die damit einhergehende Änderung des jeweiligen Sauerstoffsättigungsgrades. Die Ergebnisse zeigen, dass die hemmende Wirkung höherer Salzgehalte weitgehend oder vollständig durch Erhöhung der Menge des gelösten Sauerstoffs aufgehoben werden kann.

Alle Versuchszahlen deuten darauf hin, dass die Wichtigkeit des Salzgehalts als Teil der Umwelt von *Cyprinodon macularius* nur im Zusammenhang mit einer umfassenden mathematischen Analyse voll verstanden und geschätzt werden kann.

ENVIRONMENTAL REQUIREMENTS OF EPHEMEROPTERA. Justin W. Leonard. p. 110. Much more is known about the distribution of Ephemeroptera with regard to the physical characteristics of the environment than about their reaction to variations in water chemistry, especially chemical variations introduced by human activity. While general ecological requirements of the common species are known, conditions of microhabitat have rarely been investigated; for many described species the immature stages are themselves unknown.

In North America, a considerable part of our knowledge of mayfly ecology has been gained in connection with studies of feeding habits and requirements of fishes, especially trout, and with attempts to develop and evaluate methods of environmental manipulation to improve fisheries.

In certain parts of the country, studies of nymphal distribution made 3 or 4 decades ago have made it possible to evaluate the effect of both gradual and sudden ecological changes. In a few instances recovery of a mayfly fauna decimated by natural catastrophe, or by man-

CONDITIONS DE MILIEU DES ÉPHÉMÉROPTÈRA. Justin W. Leonard. p. 110. On connait beaucoup mieux la distribution des Éphéméroptèra en fonction des caractères physiques du milieu que leur réaction aux variations de composition chimique de l'eau, particulierement aux variations introduites par l'activité humaine. Tandis que les nécessités écologiques générales sont connues pour les espèces courantes, les conditions de microhabitat ont rarement été étudiées; pour beaucoup d'espèces décrites, les stades larvaires sont eux-mêmes inconnus.

En Amérique du Nord, une très grande partie de nos connaissances sur l'écologie des éphéméroptères a été obtenue à l'occasion d'études sur la nourriture et les conditions de vie des poissons, particulièrement de la truite et en relation avec les assais faits pour développer et évaluer les méthodes pour l'amélioration des pêches.

En certaines régions du pays, des études de distribution des nymphes effectuées il y a trois ou quatre décades ont permis d'évaluer les effets de changements écologiques soit graduels soit soudains. Dans quelques cas, la ré-

UMWELTSANSPRÜCHE DER EPHEMEROPTERA. Justin W. Leonard. p. 110. Unser Wissen über die Verteilung der Ephemeroptera hinsichtlich der physikalischen Eigenschaften ihrer Umgebung ist viel grösser als das über ihr Verhalten gegenüber Änderungen in der Chemie des Wassers, besonders solchen, die durch menschliche Tätigkeit verursacht wurden. Während die allgemeinen Ansprüche ökologischer Art für die gewöhnlichen Arten der Ephemeroptera bekannt sind, haben ihre Lebensbedingungen im Kleinen selten Interesse wachgerufen; von vielen der beschriebenen Arten sind sogar die Jugendstufen selbst noch unbekannt.

In Nordamerika sind beträchtliche Erfahrungen über die Ökologie der Eintagsfliege im Zusammenhang mit Studien über Nahrungsaufnahme und Nahrungsbedarf von Fischen, besonders Forellen, sowie in Untersuchungen zur Entwicklung und Auswertung von Verfahren, die Umwelt zum Vorteil der Fischerei zu beeinflussen, gewonnen worden.

In gewissen Teilen der Vereirigten Staaten ermöglichten Studien über das Vorkommen der Nymphen, die vor 3 bis 4 Jahrzehnten gemacht made pollution subsequently brought under control, has been followed.

An attempt is made to outline the status of our knowledge of mayfly requirements to date, and to point out problems in particular need of study.

FACTORS WHICH AFFECT THE TOLERANCE OF FISH TO HEAVY METAL POISONING. R. Lloyd. p. 181 . Recent research on the toxicity of zinc, lead, and copper salts to rainbow trout at the Water Pollution Research Laboratory, England, has been directed towards a study of the mechanism of toxic action and the effect of some chemical and physical components of the environment on their toxicity.

Histological examination of the gills of rainbow trout killed by these poisons showed that the epithelial cells of the lamellae were destroyed; no precipitation of mucus was observed.

When the log concentration of these metals is plotted against the log median period of survival of rainbow trout, the relation between them is curvilinear and, after a period of time, the curve becomes parallel to the survival time axis. This indicates that a lethal threshold concentration exists which will kill only 50 percent of a population of rainbow trout even after prolonged exposure, and that variations in the susceptibility of these fish to heavy metal poisoning caused by differences in the chemical and physical nature of the environment are best measured by the change in the lethal threshold concentration.

For zinc, lead, and copper, the log lethal threshold concentration increases linearly with the log total hardness of the water, although the slopes of the lines obtained with different metals are not the same. Although an increase in temperature reduces the survival time of rainbow trout in zinc solutions, the lethal threshold concentration does not vary to an appreciable extent. A reduction in the dissolved oxygen content of the water reduces the lethal threshold concentration of

installation d'une faune d'éphémeres, décimée par une catastrophe naturelle ou par une pollution humaine éliminée ensuite, a pu être suivie.

Un essai est fait pour tracer l'état de nos connaissances actuelles sur les besoins des éphéméroptères et pour souligner les problèmes nécessitant particulièrement une étude.

FACTEURS AFFECTANT LA TOLÉRANCE DES POISSONS À L'EMPOISONNEMENT PAR LES MÉTAUX LOURDS. R. Lloyd. p. 181. Au Laboratoire des Recherches sur la pollution de l'eau, Angleterre, les travaux récents sur la toxicité des sels de zinc, de plomb et de cuivre pour la truite arc-en-ciel ont été orientés vers l'étude de mode d'action des substances toxiques et de l'effet de certains facteurs chimiques et physiques du milieu sur leur toxicité.

On a examiné les truites arc-en-ciel dont la mort a été causée par ces poisons. L'examen histologique de leurs branchies a révélé que les cellules épithéliales des lamelles avaient été détruites; on n'a observé aucune précipitation du mucus.

Lorsque le logarithme de la concentration de ces métaux est rapporté sur un graphique avec le logarithme du mode du temps de survie de la truite arc-en-ciel, on obtient une ligne courbe qui après un certain temps devient parallèle à l'ordonée du temps de survie. Ceci indique qu'il existe un seuil des concentrations fatales qui causera la mort de seulement 50% d'une population de truits arc-en-ciel, même après une exposition prolongée. Cette courbe indique aussi que les variations de la sensibilité de ces poissons à l'empoisonnement par les métaux lourds, variations qui sont causées par les différences dans la composition chimique et physique du milieu, sont mieux mesurées par le changement du seuil des concentrations fatales.

Pour le zinc, le plomb et le cuivre, le logarithme de la limite de concentration augmente directement avec la logarithme de la dureté de l'eau, bien que la pente des segments de droites obtenues sur le graphique ne soit pas la même pour

worden waren, die Auswertung der Wirkung allmählicher und plötzlicher ökologischer Veränderungen. In einigen Fällen konnten solche Studien die Erholung einer Eintagsfliegenfauna verfolgen, welche durch Naturereignisse oder durch vorübergehende Verunreinigung infolge menschlicher Tätigkeit zahlenmässig stark zurückgegangen war.

Der Stand unserer Kenntnisse über die Umweltansprüche der Eintagsfliege wird dargestellt und Fragen, die weiteren Studiums bedürfen, werden aufgezeigt.

UMSTÄNDE, WELCHE DIE TOLERANZ VON FISCHEN GEGENÜBER VERGIFTUNGEN DURCH SCHWERMETALLE BEEINFLUSSEN. R. Lloyd. p.181. Kürzlich unternommene Untersuchungen über die Giftigkeit von Zink -, Blei - und Kupfersalzen gegenüber der Regenbogenforelle am Water Pollution Research Laboratory, Stevenage, England gingen in Richtung eines Studiums des Mechanismus der Giftwirkung und des Einflusses gewisser chemischer und physikalischer Umweltfaktoren auf die Giftigkeit.

Die Gewebeuntersuchung der Kiemen von Regenbogenforellen, welche durch diese Gifte getötet worden waren, zeigte, dass die Epithelzellen der Lamellen zerstört waren; Schleimabsonderung wurde nicht beobachtet.

Wenn die Logarithmenwerte der Konzentration dieser Metalle gegen die Logarithmen der mittleren Überlebenszeiten von Regenbogenforellen zeichnerisch dargestellt werden, so erhält man eine gekrümmte Linie, die nach einer gewissen Zeit in eine Parallele zur Überlebenszeitachse übergeht. Das bedeutet, dass es einen Schwellenwert derjenigen Todesgabe gibt, welche nur 50% einer Regenbogenforellenbevölkerung sogar nach langer Einwirkung töten würde, ferner, dass Änderungen in der Empfindlichkeit dieser Fische gegen Schwermetallvergiftungen, welche durch Unterschied in der chemischen und physikalischen Natur der Umwelt bedingt waren, am besten durch die Änderungen des Schwellenwertes der Todesgabe gemessen werden.

Für Zink, Blei und Kupfer steigt der Logarithmus des Schwellenwertes der Todesgabe linear mit dem Logarithmus der Gesamthärte des Wasser an, aber der Neigungswinkel der Geraden ist für jedes

these poisons, and the extent of the reduction is approximately the same for each metal; a hypothesis has been constructed to correlate the observed increase in toxicity with the increased flow of respiratory water through the gills. Similarly, a greater activity on the part of the fish increases its susceptibility to poisons by increasing respiratory flow.

For rainbow trout, the lethal threshold concentration of a mixture of copper and zinc salts, in either hard or soft water, is achieved when the sum of the concentrations of the individual metals, expressed as proportions of their individual lethal threshold concentrations, equals unity. It has been found that this method of summing toxicities also applies to mixtures of ammonia and zinc. Recent experiments on the toxicity of sewage effluents, which can contain zinc, copper, and ammonium salts, have shown that in the majority of the tests made, predictions of the toxicity of the effluents from their chemical analysis were in close agreement with their observed toxicities.

chacun des métaux. Dans les solutions de zinc quand l'élévation de la température diminue le temps de survie de la truite le seuil des concentrations fatales ne varie pas de facon appréciable. Une diminution du contenu en oxygène dissous dans l'eau fait baisser le seuil des concentrations fatales de ces poisons. La baisse du seuil est approximativement la même pour chaque métal. On a fait une hypothèse pour établir une corrélation entre l'augmentation observée dans la toxicité et l'augmentation de la quantité d'eau passant par les branchies pour la respiration. D'une manière semblable, une plus grande activité de la part du poisson augmente sa sensibilité aux poisons par une plus grande quantité d'eau passant par les branchies.

Pour la truite arc-en-ciel, en eau douce ou en eau dure, le seuil des concentrations fatales d'un mélange de sels de cuivre et de zinc est atteint lorsque la somme des concentrations de chacun des métaux égale l'unité. Ces concentrations sont sous forme de proportions du seuil des concentrations fatales individuelles. On a aussi trouvé que cette méthode d'addition des toxicités s'applique aussi aux mélanges d'ammoniaque et de zinc. Des travaux récents effectués sur la toxicité des eaux d'égout, qui peuvent contenir du zinc et du cuivre et des sels d'ammonium, ont démontré que dans la majorité des ces étudiés les prédictions de toxicité basées sur les analyses chimiques se rapprochent bien près des toxicités observées.

Metall ein anderer. Obwohl ein Temperaturanstieg die Überlebenszeit der Regenbogenforelle in Zinklösung vermindert, so ändert sich doch die Grösse des Schwellenwertes der Todesgabe nicht merklich. Eine Verminderung der Konzentration des im Wasser gelösten Sauerstoffs verringert den Schwellenwert der Todesgabe dieser Gifte; ferner ist grössenweise die Verminderung ungefähr die gleiche für jedes der drei Metalle. Eine Hypothese wurde aufgestellt, welche die beobachtete Zunahme der Giftigkeit mit dem vermehrten Durchfluss des Atemwassers durch die Kiemen in Zusammenhang zu bringen sucht. In ähnlicher Weise vermehrt grössere Aktivität des Fisches seine Empfindlichkeit gegen Gifte wegen der Zunahme der Menge des Atmungswassers.

Für die Regenbogenforelle wird der Schwellenwert der Todesgabe einer Mischung von Kupfer - und Zinkalzen in hartem oder in weichem Wasser dann erreicht, wenn die Summe der Konzentrationen eines jeden einzelnen dieser Metalle (ausgedrückt als Verhältnisse ihrer Schwellenwerte der Todesgaben zueinander) gleich eins ist. Es wurde gefunden, dass dieses Verfahren, die Giftigkeiten zusammenzuzählen, auch auf Mischungen von Ammoniak und Zink anwendbar ist. Versuche, welche kürzlich bezüglich der Giftigkeit von Abwässern, die Zink -, Kupfer - und Ammoniumsalze enthalten können, gemacht wurden, zeigten, dass in der Mehrzahl der ausgeführten Versuche Voraussagen über die Giftigkeit der Abwässer auf Grund der chemischen Analyse in guter Übereinstimmung mit den beobachteten Giftigkeiten waren.

THE INFLUENCE OF PREDATION ON THE COMPOSITION OF FRESH-WATER COMMUNITIES. T.T. Macan. p. 141. The invertebrate fauna of a fishpond that held no fish was studied for 5 years. A large population of trout was then introduced. Numbers of most invertebrates remained unchanged.

The number of planarians in a stream increased enormously after the onset of mild pollution. It is thought that their food supply was improved. Decrease of some insects is attributed to predation by the planarians.

In the littoral regions of rich lakes there are many species and individuals of Mollusca, Malacost-

INFLUENCE DE LA PRÉDATION SUR LA COMPOSITION DES POPULATIONS D'EAU DOUCE. T. T. Macan. p. 141. On a étudié pendant 5 ans la faune d'invertébrés dans un étang qui ne contenait pas de poisson. On y a ensuite introduit une large population de truites. Le nombre de la plupart des invertébrés est demeuré le même.

La nombre de planaires dans un cours d'eau semble augmenter considérablement dès qu'on y décèle une légère pollution. On croit que ceci serait dû à une amélioration dans la quantité de nourriture. La diminution du nombre de certains insectes serait causée par les planaires qui en font leur proie.

DER EINFLUSS DER NAHRUNGSVERSORGUNG AUF DIE ZUSAMMENSETZUNG DER FRISCHWASSERLEBEWELT. T. T. Macan. p. 141. Die Wirbellosenfauna eines Fischteiches, in welchem Fische nicht gediehen, wurde fünf Jahre lang beobachtet. Dann wurde eine grosse Anzahl von Forellen ausgesetzt. Die Zahl der meisten Wirbellosen blieb unverändert.

Nach einer geringen Verunreinigung stieg in einem Fluss die Zahl der Planarien ausserordentlich. Es wird angenommen, dass sich ihre Nahrungsversorgung verbessert hatte. Die Verminderung der Zahl gewisser Insekten wird auf die Nahrungsbedürfnisse der Planarien zurückgeführt.

raca, Oligochaeta, and Platyhelminthes, and comparatively few of insects. Oligotrophic lakes are characterized by the predominance of insects and relative scarcity of the other groups. It is uncertain whether the non-insect groups, many members of which have entered fresh water from the sea, are favored more by the abundant food supply or the rich ionic content of eutrophic lakes. It is suggested that they eat the eggs of insects, many of which are scattered in a haphazard way, and young active stages too, thereby bringing about the paucity of these animals. Their own eggs are generally laid in capsules, or gelatinous masses, or retained in a brood pouch.

Sur le littoral des lacs très productifs on trouve un grand nombre d'espèces et d'individus de Mollusques, Mallocostracés, Oligochètes et Plathelminthes, et comparativement peu d'insectes. Les lacs oligotrophiques sont charactérisés par une prédominance d'insectes et une rareté relative des autres groupes. Il est difficile de déterminer la cause exacte de la grande abondance des groupes non-insectes dont plusieurs sont passés de la mer à l'eau douce. Cas conditions serient dues, soit à une plus grande abondance de nourriture, soit à un riche contenu ionique des lacs eutrophiques. On croit que ces groupes se nourrissent d'oeufs d'insectes disperses ca et là et aussi de jeunes larves, ce qui amène une diminution considérable du nombre d'insectes. Par contre, leurs propres oeufs sont généralement pondus en masses gélatineuses ou capsules ou ils sont retenus dans un sac à couvée.

In den Küstengebieten von Seen, die reich an vielen Arten und Einzeltieren von Mollusca, Malacostraca, Oligochaeta und Platyhelminthes sind, findet man verhältnismässig wenige Insekten. Oligotrophische Seen sind durch das Vorherrschen von Insekten und eine relative Armut an den anderen Gruppen charakterisiert. Es ist unbestimmt, ob die Nicht - Insektengruppen, von denen viele vom Meer aus in die Frischwasser wanderten, mehr vom Nahrungsreichtum oder vom hohen Ionengehalt eutrophischer Seen begünstigt sind. Es wird vorgeschlagen, dass sie Insekteneier, deren viele wahllos verstreut sind und auch junge aktive Formen vertilgen und dadurch die Armut an diesen Tieren verursachen. Ihre eigenen Eier werden im allgemeinen in Kapseln, oder in Gallertmassen gelegt oder in Bruttaschen zurückgehalten.

PESTICIDE POLLUTION STUDIES IN THE SOUTHEASTERN STATES. H. Page Nicholson. p. 260. Investigations were begun in 1959 on pesticide pollution of surface and ground water to determine how generally this pollution occurs, its less obvious side effects on aquatic life, factors relating to the presence or absence of selected pesticides in water, how well they are removed from potable water by treatment, and what can be done to alleviate such pollution. Toxaphene and the gamma isomer of BHC were found in drainage from a 400-square mile river basin where cotton is a main crop. Water treatment failed to remove either insecticide from a municipal water supply. The maximum quantity recovered was 0.4 parts per billion of toxaphene and 0.75 parts per billion of gamma BHC. Sampling and analytical techniques were not totally efficient in recovering all that was present. Although toxaphene was present in the water nearly year around, no adverse effects were evident on aquatic life.

In a study of parathion in a peach orchard, that insecticide was shown to survive for at least nine months in the soil, and to reach a farm pond by means of runoff with rain water and directly as wind-blown spray. Again no adverse effects on aquatic life were demonstrated from several month's exposure where the maximum recovered concentration was 1.2 parts per billion. Chironomid larvae, however, were

ÉTUDES DES POLLUTIONS PAR PESTICIDES DANS LES ETATS DU SUD-EST. H. Page Nicholson. p. 260. Des recherches furent entreprises en 1959 sur la pollution des eaux de surface et des eaux souterraines par les pesticides. Il s'agissait de déterminer comment survient cette pollution, ses effets les moins évidents sur la vie aquatique, les facteurs relatifs à la présence ou à l'absence de pesticides sélectionnés dans l'eau, jusqu'à quel point ces crops sont éliminés de l'eau potable par traitement et ce qui peut être fait pour diminuer une telle pollution. Le Toxaphène et l'isomère gamma du BHC ont été trouvés dans les eaux de drainage d'un bassin de 400 milles carré où le coton est la culture principale. Les traitements de l'eau dans une station d'alimentation municipale ne peuvent éliminer aucun insecticide. Les quantités maximum trouvées furent 0.4 parties par billion de Toxaphène et 0.75 parties per billion de BHC gamma. Mais l'échantillonnage et les techniques d'analyse n'étaient pas suffisamment efficients pour retrouver tout ce qui était présent. Quoique le toxaphene soit présent depuis près d'un an aucun effet contraire n'était apparent pour la vie aquatique.

Dans l'étude du parathion dans un verger à pêches, cet insecticide a subsisté au moins neuf mois dans le sol, ainsi que dans une mare de ferme atteinte par les eaux de ruissellement et par les poussières poussées par le vent. Là encore, aucun effet contraire n'a été constaté

STUDIEN ÜBER WASSERVERUNREINIGUNG DURCH SCHÄDLINGSBEKÄMPFUNGSMITTEL IN DEN SÜDÖSTLICHEN STAATEN DER U.S.A. H. P. Nicholson. p. 260. Im Jahre 1959 wurden Studien über die Verunreinigung von Oberflächen - und Grundwasser durch Schädlingsbekämpfungsmittel unternommen; es sollte festgestellt werden, wie weit die Verunreingung verbreitet ist, welche weniger in die Augen fallenden Nebenwirkungen sie auf das Leben im Wasser ausübt, welche Faktoren das Vorkommen oder die Abwesenheit gewisser ausgewählter Schädlingsbekämpfungsmittel im Wasser bedingen, zu welchem Grade ihre Beseitigung durch Behandlung des Trinkwassers möglich ist und schliesslich, was zur Verminderung der Verunreinigung vorgenommen werden kann. Toxaphen und das gamma - Isomer von BHC (1,2,3,4, 5, 6 - Hexachlorcyclohexan) wurden in der Entleerung eines etwa 1000 Quadratkilometer grossen Flussbeckens gefunden, in welchem hauptsächlich Baumwolle geerntet wird. Keine dieser beiden Substanzen konnte durch Behandlung des Wassers einer städtischen Wasserversorgungsanlage entfernt werden. Die Höchstmenge, die wieder zurückgewonnen werden konnte, war 0,4 Teile/Milliarde Toxaphen und 0,75 Teile/Milliarde gamma BHC. Probenahme und analytische Untersuchungsverfahren waren nicht fähig, die Gesamtmengen der Schädlingsbekämpfungsmittel in den Proben zu erfassen. Obwohl Toxaphen fast während des ganzen Jahres im Was-

reduced in number of exposure of the adult population to spray in the terrestrial environment. The effects of an industrial spill of parathion were evident in a major river 100 miles downstream from the point of discharge.

Occurrence in surface water of pesticides in quantities below the level acutely toxic to aquatic life may be rather common in areas where pesticides are used routinely. The threshold below which harm is caused by such pollution remains to be determined since evaluation of chronic exposure effects at low levels is very difficult. More work in this area is critically needed.

sur la vie aquatique après plusieurs mois d'exposition à une concentration maximum de 1.2 parties per billion. Cependant les larves de chironomedes étaient moins nombreuses parès exposition des adultes à une vaporisation sur les terres environnantes. Les effets d'un épandage industriel de parathion furent évidents dans une grande rivière, 100 milles à l'aval du point de déversement.

La présence de pesticides dans les eaux de surface, en concentration inférieure au niveau rapidement nocif, peut être assex fréquente dans les régions où des pesticides sont couramment utilisés. Le seuil au dessous duquel un effet dangereux est provoqué par une telle pollution reste à déterminer puisque l'estimation des effets d'une exposition continue à de faibles concentrations est très difficile. Des travaux complémentaires sont nécessaires en ce domaine.

ser gefunden wurde, so wurden doch keinerlei Beweise für seine ungünstigen Einflüsse auf im Wasser lebende Pflanzen oder Tiere gefunden.

In einem Pfirsichgarten konnte Parathion (o,o-diathyl-o, p-nitrophenylphosphorothioat) noch nach 9 Monaten im Boden nachgewiesen werden; auch konnte gezeigt werden, dass es sowohl in abfliessendem Regenwasser als auch durch Windzerstäubung den Weiher in einem Bauernhof erreicht hatte. Auch hier konnten nach mehreren Monaten keine ungünstigen Einflusse auf das Leben im Wasser festgestellt werden, trotzdem die zurückgewonnene Höchstkonzentration 1,2 mg/l betrug. Die Zahl von Chironomid Larven ging aber zurück, wenn die erwachsenen Formen auf dem Lande der Zerstäubung ausgesetzt waren. In einem grösseren Fluss zeigten sich die Wirkungen eines industriellen Überlaufs, der Parathion enthielt, noch 160 km stromabwärts der Einleitungsstelle.

Das Vorkommen von Schädlingsbekämpfungsmitteln in Oberflächenwasser in Mengen unter den für Wasserorganismen akut giftigen dürfte in Gegenden, wo diese Stoffe laufend verwendet werden, ziemlich allgemein sein. Der Schwellenwert, unterhalb dessen eine derartige Verunreinigung schädlich wirkt, muss erst noch bestimut werden, da die Auswertung von chronischen Wirkungen kleiner Mengen sehr schwierig ist. Mehr arbeit auf diesem Gebiet ist dringend nötig.

ALGAE AS INDICATORS OF POLLUTION. Ruth Patrick. p. 225. During the twentieth century a great deal of literature has accumulated pertaining to algae as indicators of pollution. The word "pollution" is a collective noun referring to many chemical and physical changes that may occur in a body of water due to the activities of man. The first kind of pollution recognized was organic in nature. Species were classified as to the degree of organic pollution they could tolerate. Little attention was given to the sizes of the population of the various species or to the structure of the algae community as a whole. The presence or absence of a few or several species were used to indicate the degree of pollution. Further work showed that this method of indicating pollution often was misleading. At about the same time, several workers developed a system of indicating pollution by the kinds of species that were dominant in an algal community. Several examples

LES ALGUES CONSIDÉRÉES COMME INDICATEURS DE POLLUTION. Ruth Patrick. p. 225. Depuis le début du vingtième siècle, de nombreus articles ont paru concernant les algues comme indicateurs de pollution. Le mot "pollution" étant un nom collectif désignant les nombreux changements chimiques et physiques pouvant apparaître dans une eau par suite des activités humaines. La première sorte de pollution reconnue était d'origine organique. Les espèces furent classées selon le degré de pollution organique qu'elles pouvaient tolérer. Peu d'attention fut apportée à l'importance des populations des différentes espèces où à la structure de la communauté algale dans son ensemble. La présence ou l'absence de quelques espèces ou de plusieurs espèces servait à indiquer le degré de pollution. Des travaux ultérieurs ont montré que cette méthode était souvent erronée. A peu près en même temps plusieurs chercheurs

ALGEN ALS LEITORGANISMEN DER WASSERVERUNREINIGUNG. Ruth Patrick. p. 225 .Im Laufe des 20. Jahrhunderts sind zahlreiche Arbeiten über Algen als Leitorganismen der Wasserverunreinigung erschienen. Das Wort „Verunreinigung" ist ein Sammelwort für die vielen chemischen und physikalischen Veränderungen, welche in einem Wassersystem infolge menschlicher Tätigkeit vorkommen können. Die erste Art von Verunreinigungen, die als solche erkannt wurde, war ihrem Wesen nach organisch. Man unterschied die Arten von Organismen nach dem Grade der organischen Verunreinigung, welche von ihnen ertragen wurde. Wenig Aufmerksamkeit wurde der Grösse der Bevölkerung der verschiedenen Arten oder dem Aufbau der Algenflora als Ganzem geschenkt. Die Anwesenheit oder Abwesenheit einer geringen Zahl von Algen oder einiger weniger Arten zeigte den Grad der Verunreinigung an. Spätere Arbeiten zeigten, dass are given in which this method was used.

With the increased complexity of civilization the word "pollution" included a greater number of substances that had very different chemical and physical characteristics. Thus, the general use of indicator species become much more difficult. It has been shown further that variations in the natural environment may greatly influence the size of the populations of "indicator species" and even their occurrence. Thus, well established populations of several species of algae may indicate the presence of certain types of pollution, but a decrease in the sizes of their population or their absence may be due to very different causes than a change in the amount of pollution present.

In recent years the importance of considering the structure of the whole algae community as well as the kinds of species composing it has been pointed out by several workers. Examples are given as to the ways in which various kinds of pollution may affect the structure of algae communities and the kinds of species composing them.

développèrent un système où la pollution était décelée d'après les espèces dominantes dans la communauté algale. Plusieurs exemples sont donnés en utilisant cette méthode.

Avec la crossante complexité de la civilisation, le mot « pollution » englobe un plus grand nombre de substances qui ont des caractéristiques chimiques et physiques très différentes, aussi l'emploi général d'espèces indicatrices divient beaucoup plus difficile. Il a été montré que des variations du milieu naturel peuvent influencer énormément l'importance de la population des espèces dominantes et même leur présence. Aussi des populations bien établies de plusieurs espèces d'algues peuvent indiquer la présence de certains types de pollution, mais une diminution de quantité ou même leur disparition peuvent être dues à des causes autres qu'un changement dans la pollution présente.

Ces dernières années plusieurs chercheurs ont souligné l'importance de considérer la structure de toute la communauté algale aussi bien que les espèces la composant. Des exemples sont donnés sur la manière dont ces éléments peuvent être affectés par différentes sortes de pollution.

dieses Verfahren oft irreführend war. Etwa zur gleichen Zeit entwickelten verschiedene Forscher eine Methode, welche den Verschmutzungsgrad durch die in der Algenflora vorherrschenden Arten anzeigte. Einige Beispiele der Anwendung dieses Verfahrens werden besprochen.

Gleichlaufend mit dem immer verwickelter werdenden Aufbau unserer Zivilisation musste auch das Wort „Verunreinigung" eine immer grössere Zahl von Stoffen, die sich in ihren chemischen und physikalischen Eigenschaften sehr voneinander unterschieden, umfassen. Dadurch wurde der allgemeine Gebrauch von Leitorganismen viel schwieriger. Ausserdem lernte man, dass Veränderungen in der natürlichen Umgebung die Zahl der Leitorganismen und manchmal sogar ihr Vorkommen sehr wesentlich beeinflussen können. So mögen z. B. mehrere Arten Algen in gut wachsenden Beständen die Anwesenheit gewisser Formen von Verunreinigungen anzeigen; jedoch könnte eine Verminderung der Grösse der Algenflora, oder gar ihre Abwesenheit durch viele andere Ursachen als durch einen Wechsel in der Menge der Verunreinigungen hervorgerufen werden.

Seit kurzem wird von verschiedenen Forschern die Wichtigkeit des Aufbaus der gesamten Algenflora und der verschiedenen Arten in ihr betont. Beispiele dafür, wie die verschiedenen Arten von Wasserverunreinigung den Aufbau der Algenflora und der in ihr auftretenden Arten beeinflussen können, werden gegeben.

ACCUMULATION OF CESIUM-137 THROUGH THE AQUATIC FOOD WEB. Robert C. Pendleton. p. 355. Cesium-137 is more available to organisms living in aquatic environments than in terrestrial environments; consequently, foods of man that come from aquatic or wet-land sources may contain much more of this isotope than foods from normal cropland. The increase in availability in aquatic situations results from movement of the cesium ions through water directly to the plant surfaces before they are immobilized by mud or other non-living surfaces. Concentrations of cesium-137 in aquatic animals are directly related to the trophic levels, with the highest levels occurring in the flesh of predators. The content of cesium-137 in tissues of omnivores lies between that of herbivores and

ACCUMULATION DE CÉSIUM 137 DANS LES TISSUS D'ANIMAUX AQUATIQUES. Robert C. Pendleton. p. 355. Le césium 137 est plus fréquemment recontré dans les organismes vivants dans des milieux aquatiques que dans des milieux terrestres; en conséquence, la nourriture, consommée par l'homme venant de source aquatique ou de marais peut contenir beaucoup plus de cette isotope que les nourritures venant d'organisme terrestre. L'accroissement de cette disponibilité dans les situations aquatiques résulte du mouvement des ions du césium à travers l'eau jusqu'aux plantes de surface avant qu'ils soient arrêtés par le boue ou d'autre organes morts. Les concentrations de césium 137 dans les animaux aquatiques sont directement en rapport avec les niveau trophic

ANREICHERUNG VON CAESIUM-137 DURCH DEN NAHRUNGSKREISLAUF IM WASSER. Robert C. Pendleton. p. 355. Caesium-137 ist für im Wasser lebende Organismen leichter zugänglich als für Landorganismen; folglich können menschliche Nahrungsmittel, die aus dem Wasser oder aus feuchten Landstrichen stammen, mehr von dieser Atomart enthalten als solche von normalem Ernteland. Die grössere Zugänglichkeit aus feuchten Lagen ergibt sich aus der Beweglichkeit der Caesiumionen durch das Wasser unmittelbar zu den Pflanzenoberflächen, ehe sie durch Schlamm oder andere nicht - lebende Stoffe unbeweglich gemacht werden. Die Caesium-137 Konzentration in Wassertieren hängt unmittelbar mit der Ernährungsstufe zusammen und die höchsten Konzen-

predators. The stepwise increase of cesium-137 concentration in the successive trophic levels is related to the difference in the biological half-lives of cesium and potassium. Because the biological half-life of cesium is about three times longer than that of potassium, the organism comes to a steady state at a contamination level for cesium greater than the level in its food. Predacious fish may accumulate cesium-137 to 10,000 times the amount in water, and a comparison of concentration factors and yield per hectare of animal products from grazing lands and fish farming indicates a greater potential hazard from the latter. High availability of cesium-137 to aquatic and emergent plants indicates that milk and meat from grazing animals utilizing forage from wetlands may contain much more cesium-137 from fallout than such products from drylands. Thus, observed geographic variations in cesium-137 content of milk may be in part a result of different levels of uptake by plants of wet and of dry lands. Because environmental variations cause great differences in uptake of cesium-137, evaluation of the hazard to man from this isotope should be made on local rather than regionwide bases. Hazards evaluation, as presented, is limited to freshwater conditions.

avec les niveaux le plus élevé ayant lieu dans la chair des prédatores. Le contenu de césium 137 dans les tissues des omnivores se situent entre celui des herbivores et des prédatores. L'accroissement de concentration de césium 137 dans les niveaux trophiques successifs est en rapport avec la différence de la moitié de vie biologique du césium et du potassium. Du fait que la moitié de vie biologique du césium est environ trois fois plus grande que celle du potassium, l'organisme vient a un état sur au niveau de concentration pour le césium plus grand que le niveau de la nourriture. Le poisson de proie peut accumuler 10.000 fois plus de césium 137 que l'eau et une comparaison de facteur de concentration par hectare de produits d'animaux, venant de pâturage et de vivier indique un plus grand potentiel de chance pour le dernier nommé. La haute disponibilité de césium 137 sur les plantes aquatiques et de surface indique que le lait et la viande venant de bovins bubant de l'eau forée dans les marias peuvent contenir beaucoup plus de césium 137 que les mêmes produits venant de pâturages normaux. Aussi, les variations géographiques observé dans le pourcentage de césium 137 dans le lait peut être en partie un résultat des niveaux differents d'absorption par les plantes de terrains humides et secs. Du fait que les variations du milieu cause de grande différence dans l'aptitude d'absorber du césium 137, l'évaluation du danger pour l'homme à l'encontre de cet isotope serait faite sur des regions locales plutôt que sur des regions de base. L'évaluation des dangers ainsi presentée est limitée aux conditions d'eau douce.

trationen werden in Fleisch von räuberisch lebenden Tieren gefunden. Der Caesium-137 Gehalt der Gewebe von Omnivoren liegt zwischen dem der Herbivoren und der räuberisch lebenden Organismen. Die stufenweise Zunahme der Caesium-137 Konzentration in den aufeinanderfolgenden Ernährungsstufen steht mit dem Unterschied in den biologischen Halbwertszeiten von Caesium und Kalium in Zusammenhang. Weil diese Halbwertszeit für Caesium ungefähr dreimal so lang wie die des Kaliums ist, so kommt ein Organismus bei höheren Verschmutzungen durch Caesium Früher zum Gleichgewichtszustand als der Gehalt an diesem Element in seiner Nahrung andeuten würde. Raubfische können Caesium-137 aus Wasser auf das 10 000fache anreichern und ein Vergleich von Konzentrationsfaktoren und Ausbeute auf je ein Hektar an tierischen Produkten von Weideland und Fischzucht weist auf die letztere als die grössere mögliche Gefahrenquelle hin. Weil Caesium-137 untergetauchten und teilweise herausragenden Wasserpflanzen leicht zugänglich ist, so ist zu vermuten, dass Milch und Fleisch von Tieren, die auf feuchtem Land weiden, viel mehr Caesium-137 aus radioaktivem Ausfall enthalten als die gleichen Produkte aus trockenen Weiden. Es ist möglich, dass die geographischen Verschiedenheiten des Caesium-137 Gehaltes von Milch wenigstens zum Teil auf Unterschiede in der Aufnahme dieses Elements durch Pflanzen auf feuchten und auf trokkenen Böden zurückgefuhrt werden können. Da Umgebungsänderungen grosse Unterschiede in der Caesium-137 Aufnahme verursachen können, so sollten Auswertungen der möglichen Gefahren von dieser Elementart für den Menschen auf Grund der örtlichen Verhältnisse und nicht im Bezug auf grosse Flächenbereiche gemacht werden. Die Gefahrenauswertungen des Vortragenden beschränken sich auf die Bedingungen in Süsswasser.

THE EFFECTS OF STREAM SEDIMENTATION ON TROUT EMBRYO SURVIVAL. John Peters. p. 275. Five sampling stations were established in Bluewater Creek to measure sediment concentrations and discharge. In the vicinity of the sediment-discharge stations, manmade redds were constructed with sorted gravel, and eyed rainbow trout eggs in hatching boxes were introduced into the redds. Periodically, the Mark VI standpipe apparatus was used to measure

INFLUENCE DE LA SÉDIMENTATION EN RUISSEAU SUR LA SURVIVANCE DES EMBRYONS DE TRUITES. John Peters. p. 275. Cinq stations de prélévement furent choisies dans la Bluewater Creek pour mesurer les concentrations en sédiment et les déversements. Au voisinage des déversements chargés de sédiment, des frayères artificielles furent aménagées avec des graviers triés, et des oeufs embryonnés de truite arc-en-ciel, placés dans des boites à incubation,

DIE WIRKUNGEN DER ABSETZUNGSVORGÄNGE IN FLÜSSEN AUF DAS ÜBERLEBEN VON FORELLENEMBRYONEN. John Peters. p. 275. Zum Zwecke der Messung von Bodensatzkonzentrationen und Ablaufmenge wurden im Bluewater Creek fünf Untersuchungsstellen gewählt. In ihrer Nähe wurden mit gesiebtem Kies Laichplätze errichtet und beaugte Regenbogenforelleneier in Kästen an diesen Stätten ausgesetzt. Von Zeit zu Zeit wurden mit dem Mark VI intragravel dissolved oxygen and intragravel apparent velocity within the redds. The sampling stations with low sedimentation rates responded with high intragravel dissolved oxygen rates, high intragravel seepage rates (apparent velocities), and low trout embryo mortality. Conversely, the sampling stations with high sediment rates responded with low intragravel dissolved oxygen rates, low intragravel seepage rates (apparent velocities), and high trout embryo mortality.

furent introduits sur ces frayères. Périodiquement, l'appareil à colonne ascendante Mark VI était utilisé pour mesurer l'oxygène dissous et la vitesse apparente dans les graviers de la frayère. Les stations à faible vitesse de sédimentation correspondaient à un taux d'oxygène dissous élevé dans les graviers, à une infiltration rapide (vitesse apparente) et à une faible mortalité des embryons de truite. Inversement, les stations à vitesse de sédimentation rapide correspondaient à de faibles teneurs en oxygène dissous dans les graviers, à une infiltration lente et à une forte mortalité chez les embryons.

Standrohrgerät im Kiesbett Messungen des gelösten Sauerstoffs und der scheinbaren Durchflussgeschwindigkeit in den Laichplätzen gemacht. An Untersuchungsstellen mit niedriger Absetzgeschwindigkeit wurden hohe Werte für gelösten Sauerstoff, hohe Sickerungsgeschwindigkeit und niedrige Sterblichkeit der Forellenembryonen gefunden. Umgekehrt hatte hohe Absetzgeschwindigkeit niedere Werte für gelösten Sauerstoff, niedere Sickerungsgeschwindigkeit (scheinbare Durchflussgeschwindigkeit) und hohe Sterblichkeit der Forellenembryonen zur Folge.

ACCUMULATION OF RADIONUCLIDES AND THE EFFECTS OF RADIATION ON MOLLUSCS. Thomas J. Price. p. 202 . The accumulation and retention of cesium-137, cerium-144 zinc-65, and gold-199 by the hard clam, *Mercenaria mercenaria*, by the bay scallop, *Aequipecten irradians*, and by the oyster, *Crassostrea virginica*, were determined in laboratory experiments. Experiments with cesium-137 and cerium-144 were carried out with single species held in separate holding containers. All of these animals concentrated cesium-137 over the amounts occurring in the sea water. A more rapid uptake was found for the soft tissues other than muscle. The highest concentrations were reached in time, however, by muscle tissue. Uptake and loss of cesium-137 proceeded at different rates with time, being more rapid initially followed by a slower continued rate. The accumulation of cerium-144 by clams and their separated shells indicates that the physical state of this isotope in sea water is an influencing factor. Cerium-144 occurs mainly as particles in sea water and was taken up by the molluscs directly from the water or obtained from materials that had been associated with phytoplankton. This radionuclide was accumulated by adsorption of the particles to body surfaces of the clam or by retention in the organs and structures connected with the digestive tract. The initial loss of cerium-144 by clams and scallops was rapid followed by a reduced rate of loss. After 195 days, 20 percent of the original cerium-144 remained in the clams. Of this, 11 percent was found in the visceral mass, 38 percent in the muscle, and 45 percent in the shell. After 35 days, shells of scallops had lost 55 per-

ACCUMULATION DES RADIONUCLIDES ET EFFETS DE LA RADIATION SUR LES MOLLUSQUES. Thomas J. Price. p. 202. On a déterminé par des essais de laboratoire l'accumulation et la rétention de césium-137, de cérium-144, de zinc-65, et d'or-199 chez les palourdes, *Mercenaria mercenaria*, les pétoncles de baie, *Aequipecten irradians*, et les huîtres *Crassostrea virginica*. Au cours des expériences avec le césium-137 et le cérium-144, on a utilisé des contenants séparés pour une espèce seule. Tous ces animaux ont concentré le césium-137 en quantités plus grandes que celles qu'on trouve dans l'eau de mer. On a trouvé que cette accumulation est plus rapide dans les tissus mous, autres que les muscles. Toutefois, avec le temps, les plus fortes concentrations ont été atteintes par les tissus musculaires. L'accumulation et la perte de césium-137 se produisent à des vitesses différentes suivant le temps; au début il y a une phase rapide suivie d'une phase plus lente mais soutenue. L'accumulation du cérium-144 par les palourdes et leurs coquilles séparées indique que l'état physique de cet isotope dans l'eau de mer est un facteur qui influence cette accumulation. Le cérium-144 se présente surtout sous forme de particule dans l'eau de mer où les mollusques l'ont pris directement ou bieu l'ont obtenu d'organismes qui sont associés au phytoplancton. L'accumulation de ce radionuclide s'est fait par adsorption des particules à la surface extérieure des palourdes ou par leur rétention dans les organes et les structures associées au système digestif. La perte de cérium-144 par les palourdes et les pétoncles est rapide au début puis ensuite plus lente. Après 195 jours, il ne reste dans

ANREICHERUNG VON RADIOAKTIVEN ELEMENTEN UND STRAHLUNGSEINFLUSSE AUF MOLLUSKEN. Thomas J. Price. p. 202. Die Anreicherung und Speicherung von Caesium-137, Cer-144, Zink-65 und Gold-199 durch die Venusmuschel, *Mercenaria mercenaria*, die Bucht - Kammuschel, *Aequipecten irridans*, und durch die Auster, *Crassostrea virginica* wurde in Laboratoriumsversuchen bestimmt. Die Versuche mit Caesium-137 und mit Cer-144 wurden mit jeweils einer Art in getrennten Behältern durchgeführt. Alle Tiere reicherten Caesium-137 gegenüber den im Meerwasser vorkommenden Beträgen an. Für die Weichteile wurde eine raschere Aufnahme festgestellt als für Muskelfleisch, letzteres erreichte jedoch mit der Zeit die höchsten Konzentrationen. Aufnahme und Verlust von Caesium-137 gingen mit verschiedenen Geschwindigkeiten vor sich: die anfangs rasche Aufnahme verlangsamte sich und ging schliesslich in die Abgabe über. Die Anreicherung von Cer-144 durch Muscheln und durch Muschelschalen zeigte, dass der physikalische Zustand dieser Atomart im Meerwasser von Einfluss ist. Cer-144 ist im Meerwasser in festen Teilchen zu finden; es wurde von den Mollusken unmittelbar aus dem Wasser aufgenommen oder aus Stoffen erhalten, die vorher an Phytoplankton gebunden waren. Dieses Radioelement wurde durch Adsorption der Teilchen an Körperoberflächen der Muschel oder durch Speicherung in den Verdauungsorganen und im Verdauungskanal angereichert. Der anfängliche Verlust der Venus - und der Kammuscheln an Cer-144 was anfangs beträchtlich und nahm dann ab. Nach 195 Tagen waren nur noch 20 v. H. des ursprünglich vorhandenen Cer-144 in den Muscheln verblieben;

cent of their initial activity, adductor muscle, 62 percent, and visceral mass, 89 percent.

The accumulation of zinc-65 and gold-199 by clams, separated clam shells, and oysters was followed in an experimental marine community that also contained fish, crabs, and sediments. A comparison of the uptake of zinc-65 by clams and oysters revealed that after 21 days oysters had accumulated the nuclide approximately 8 times over that in clams; however, there was no indication that maximum levels of zinc-65 had been reached in either organism. Live clams removed 3.6 times more gold-199 than separated clam shells. Clams that had burrowed in montmorillonite clay at the bottom of the tank contained 38 percent less gold-199 than others remaining in the radioactive sea water.

Experiments are in progress to determine the effects of gamma radiation from a high level cobalt-60 source on shellfish. Preliminary observations indicate that clams are more radiosensitive than oysters to this radiation.

RADIOACTIVITY IN FRESHWATER ORGANISMS OF THE LAKES OF NORTHERN ITALY. Oscar Ravera. p. 195. Samples of plankton, larger aquatic plants, fishes, benthos, molluscs, and sediment collected in some lakes of northern Italy were examined to compare their radioactivity. Manganese-54, a gamma emitting activation product, was found in the soft tissues of the lamellibranchs. The radionuclide content is very different in populations settled in different zones of the same lake (Lake Maggiore). The highest level of radioactivity was found in the sediment in which the total beta-activity was observed to increase with increasing depth. The periphyton always showed higher radioactivity values than the aquatic plant on which it lives. The season and the zone have only a little influence on the uptake of radioactivity by plankton, fish, and benthos, whereas age plays a very important role in this process both in the mollusc and in the fish.

les palourdes que 20% du cérium-144 du début. On a trouvé 11% de ce cérium dans les viscères, 38% dans le muscle, et 45% dans la coquille. Après 35 jours, les coquilles de pétoncles avaient perdu 55% de leur activité initiale, le muscle adducteur 62%, et les viscères 89%.

On a suivi l'accumulation du zinc-65 et de l'or-199 chez les palourdes, leurs coquilles séparées et chex les huîtres dans un habitat marin qui comprenait aussi des poissons, des crabes, et des sédiments. En comparant l'absorption du zinc-65 par les palourdes et par les huîtres on a trouvé qu'après 21 jours les huîtres avaient accumulé 8 fois plus de cette substance que les palourdes; toutefois rien n'indique que les niveaux maxima de zinc-65 aient été atteints pour l'un au l'autre. Les palourdes vivantes absorbent 3.6 fois plus d'or-199 que les coquilles seules. Les palourdes qui s'étaient enfouies dans la glaise « montmorillonite » au fond du réservoir contenaient 38% moins d'or-199 que les autres spécimens qui étaient restés dans l'eau de mer radioactive.

On a entrepris des travaux pour déterminer l'effet de la radiation gamma provenant d'une source élevée de cobalt-60 sur les mollusques. Les observations préliminaires indiquent que les palourdes sont plus sensibles à cette radiation que les huîtres.

RADIOACTIVITÉ DES ORGANISMES D'EAU DOUCE DANS LES LACS DU NORD DE L'ITALIE. Oscar Ravera. p. 195. Des échantillons de plancton, plantes aquatiques supérieures, poissons, benthos, mollusques et sédiments recueillis dans quelques lacs du Nord de l'Italie furent examinés pour comparer leur radioactivité. Le manganèse 54, produit émetteur de rayons gamma, fut trouvé dans les tissues mous des lamellibranches. La teneur en radionucléide est très differente dans les populations établies en divers points d'un même lac (Lac Majeur). La radioactivité la plus élevée fut trouvée dans les sédiments et, dans ceux-ci, l'activité beta totale augmente avec la profondeur. La périphyton présente toujours une radioactivité plus élevée que les plantes supérieures sur lesquelles il vit. La saison et la situation ont peu d'influence sur la fixation de la radioactivité par le plancton, le poisson et le benthos tandis gue l'âge joue un grand rôle dans ce processus, à la fois chez les mollusques et les poissons.

davon waren 11 v. H. in der Eingeweidemasse, 38 v. H. im Muskel und 45 v. H. in der Schale. Nach 35 Tagen hatten die Schalen der Kammuscheln 55 v. H. ihrer ursprünglichen Aktivititat verloren, der Schliessmuskel 62 v. H. und die Eingeweidemasse 89 v. H.

Die Anreicherung von Zink - 65 und von Gold-199 durch Kammmuscheln, Muschelschalen und durch Austern wurde in einer marinen Versuchanlage, die auch Fische, Krabben und Sedimente enthielt, untersucht. Ein Vergleich der Aufnahme von Zink - 65 durch Muscheln und Austern zeigte, dass nach 21 Tagen die Austern etwa achtmal mehr radioaktives Element angereichert hatten als die Muscheln; es war aber kein Anzeichen dafür vorhanden, dass in dem einen oder anderen der Organismen Höchstwerte an Zink - 65 erreicht worden waren. Es wurde gefunden, dass lebende Muscheln 3,6 mal mehr Gold - 199 entfernten als die Muschelschalen allein. Muscheln, die sich in den Montmorillonitlehm am Versuchsbehälterboden eingegraben hatten, enthielten 38 v. H. weniger Gold - 199 als andere, die im radioaktiven Meerwasser geblieben waren.

Versuche zur Bestimmung der Wirkung starker gamma - Strahlung von Kobalt - 60 auf Schalentiere sind im Gange. Vorläufige Beobachtungen zeigen, dass Muscheln für diese Strahlung empfindlicher sind als Austern.

RADIOAKTIVITÄT IN FRISCHWASSERORGANISMEN DER NORDITALIENISCHEN SEEN. Oscar Ravera. p. 195. Proben von Plankton, grosseren Wasserpflanzen, Fischen, Benthos, Mollusken und Sediment aus einigen der norditalienischen Seen wurden auf ihre Radioaktivität untersucht und die Ergebnisse verglichen. Der gamma-Strahler Mangan - 54 wurde in den Weichteilen der Lamellibranchen gefunden. Der Gehalt an radioaktiven Elementen in den Bewohnern der verschiedenen Tiefenzonen ein und desselben Sees ist sehr unterschiedlich. Höchstwerte der Radioaktivität wurden im Sediment gefunden; in diesem zeigte sich mit zunehmender Tiefe ein Ansteigen der Radioaktivität. Das Periphyton hatte stets höhere Werte der Radioaktivität als die Wasserpflanzen, auf welchen es lebt. Jahreszeit und Zone haben nur geringen Einfluss auf die Aufnahme von Radioaktivität durch Plankton, Fische und Benthos, während in Mollusken und Fischen das Alter in diesem Vorgang eine sehr wichtige Rolle spielt.

DIATOMS AND THEIR PHYSICO-CHEMICAL ENVIRONMENT. Charles W. Reimer. p. 19 . In the past 40 years considerable field data have been gathered concerning the physical and chemical factors of the water in which certain diatoms have been found. Some of these data have been worked into key-word series, each series representing some factor of the environment, i.e., pH series, acidobiont, acidophil, indifferent, alkaliphil, alkalibiont.

Such terms have not received wide usage in this country as regards the algae. Indeed, at the species level little has been done here to indicate whether or not such ecological amplitudes, be they in actual figures or in key-word series, are generally applicable.

In this study the following diatom taxa are considered: *Cymbella tumida* (Breb.) V.H., *Melosira varians* Ag., *Navicula confervacea* Kütz., *Navicula ingenua* Hust., and *Nitzschia amphibia* Grun. The water quality ranges and ecological categories applied to these diatoms by other workers are compared to the physico-chemical characteristics of water in which these diatoms have been found in this country. Data used in this compilation represent 59 sampling series from 8 different rivers.

In general, there is good agreement between the literature reports and our data for these taxa. In many cases this is the first report on certain chemical concentrations relating to these diatom species; e.g., this is the first report of the chemical condition of the water in which the diatom, *Navicula ingenua*, was found living.

DIATOMES ET LEUR MILIEUX AMBIANTS PHYSICO-CHIMIQUES. Charles W. Reimer. p. 19 . Dans les dernières 40 années, un numbre considérable de données ont ete recueilli concernant les facteurs chimiques et physiques de l'eau dans laquelle certains diatomes ont été trouve. Quelques unes de ces données ont été organisé in series chaque serie représentant des facteurs de milieu ambiant, par exemple pH série, acidobiont, acidophile, indifferent, alkaliphile, alkalibiont.

De tels termes n'ont pas recu un large usage dans ce pays relativement a l'algue. Évidemment, relativement aux espèces, peu a été fait ici pour indiquer si de telles amplitudes écologiques étant dans des figures actuelles ou dans des classification, sont applicables.

Dans cette étude les diatome taxa suivants sont considérés: *Cymbella tumida* (Breb.) V. H., *Milosira varians* Ag., *Navicula conjervacea* Kütz., *Navicula ingenua* Hust., et *Nitzschia amphibia* Grun. Les domaines de qualité de l'eau et de catégorie écologique appliqués à ces diatomes par d'autres travaux sont comparés aux caractéristiques physico-chimiques de l'eau dans laquelle ces diatomes ont été trouvé dans ce pays. Les données utilisées dans cette compilation représentent 59 series d'échantillons de 8 différents fleuves.

En général il y a un bon agrément entre les rapports littéraires et notre donnée pour ces taxa. Dans beaucoup de cas, ceci est le premier rapport sur certaines concentrations chimiques relative à ces espèces de diatomes, c'est-à-dire, le premier rapport des conditions chimiques de l'eau dans lequel le diatome *Navicula ingenua*, a été trouvé vivant.

DIATOMEEN UND IHRE PHYSIKALISCH - CHEMISCHE UMWELT. Charles W. Reimer. p. 19 . In den verflossenen 40 Jahren wurde eine grosse Menge von Angaben über die physikalischen und chemischen Eigenschaften des Wassers, in welchem gewisse Diatomeen gefunden wurden, gesammelt. Einige dieser Werte wurden in Stichwortreihen angeordnet, wo jede Reihe einen Umgebungsfaktor, nämlich pH Stufen, säurefest, acidophil, neutral, alkaliphil, alkalifest darstellt. Eine solche Einteilung hat im Bezug auf Algen in den Vereinigten Staaten bisher nur begrenzte Anwendung gefunden. Tatsächlich wurde bezüglich der Arten nur sehr wenig getan, dass es schwer ist, festzustellen, ob solche oekologische Eigenschaften, mögen sie in Zahlen oder in Stichwortreihen vorliegen, allgemein anwendbar sind.

In der vorliegenden Studie wurden die folgenden Abteilungen von Diatomeen berücksichtigt: *Cymbella tumida* Brébisson, V. H., *Melosira varians* Agardh, *Navicula confervacea* Kützing, *Navicula ingenua* Hustedt, and *Nitzschia amphibia* Grün. Die Arten und oekologischen Bereiche der Wassergüte, welche von anderen Forschern auf diese Diatomeen angewendet wurden, werden mit den physikalisch - chemischen Eigenschaften der Gewässer verglichen, in welchen diese Diatomeen in den Vereinigten Staaten gefunden wurden. 8 verschiedene Flüsse sind in 59 Versuchsreihen vertreten.

Im allgemeinen ist die Übereinstimmung zwischen Literaturangaben und unseren Feststellungen gut. Oft berichten wir als die Ersten über bestimmte chemische Bedingungen des Wassers für bestimmte Diatomeenarten; dies ist zum Beispiel für *Navicula ingenua* der Fall.

ENVIRONMENTAL REQUIREMENTS OF TRICHOPTERA. Selwyn S. Roback. p.118 . Over many years, the Limnology Department of the Academy of Natural Sciences of Philadelphia has conducted stream surveys at over 100 stations in the United States and Canada. At any of these stations, the caddisfly larvae form about 10 percent of the total insect fauna. The ranges of occurrence for each of 14 chemical factors are given for the dominant genera, and in some cases families (where there were too few records) of caddisfly larvae. Too few of the larvae collected can be placed as to species with sufficient certainty to make data at the species level

CONDITION DU MILIEU AMBIANT DE *TRICHOPTERA*. Selwyn S. Roback. p. 118 . Depuis de nombreuses années le Départment Limnologique de l'Académie de Sciences Naturelles de Philadelphie conduit l'étude des courants d'eau sur plus de 100 places aux USA et au Canada. Dans la plupart de ces stations, les larves *Trichoptera* forment entre les 10% de la faune insecte. Les domaines de fait pour chacun des 14 facteurs chimiques sont donnés par la génération dominante, et dans des cas de famille (où il y avait trop peu de rapports) par les larves *Trichoptera*. Trop peu de ces larves collectees peuvent être placé,

DIE ANFORDERUNGEN DER TRICHOPTEREN AN IHRE UMGEBUNG. Selwyn S. Roback. p.118 . Viele Jahre lang hat das Limnology Department of the Academy of Natural Sciences of Philadelphia Untersuchungen an Flüssen an über 100 Versuchsstellen in den Vereinigten Staaten und in Kanada durchgeführt. An allen diesen Orten bilden die Eintagsfliegenlarven etwa 10% der Insektenfauna. Die Bereiche des Vorkommens für jeden einzelnen von 14 chemischen Faktoren werden für die vorherrschenden Gattungen und in einigen Fällen für die Familien (wenn nicht genügend Aufzeichnungen vorhanden waren) von Eintagsfliegenlarven gegeben. Zu wenige der

meaningful. Laboratory experiments on the toxicity of various chemicals to caddisfly larvae are practically non-existent; however, the results of one set of experiments performed at the Academy are presented.

THE APPLICATION OF DIATOM ECOLOGY AND PHYSIOLOGY TO PROBLEMS OF WATER POLLUTION AND PURIFICATION. F.E. Round. p. 29. The distribution of diatoms over the range of polluted water is discussed as well as their relation to standards of unpolluted natural waters. A suggested widening of the scope of studies of diatoms in polluted waters to include the flora of the individual habitats is outlined and the data compared with experimental methods of assessing pollution. The classification of the habitats and the methods of sampling are discussed. The basic ecological requirements of diatoms and the modification of these in polluted waters are detailed. Probable mode of action of pollutants on diatoms and the beneficial effects of the diatom flora in self-purification of polluted waters are discussed. Detection of incipient pollution from a study of lake sediments involving indicator species and the need for a re-evaluation of indicator species are argued.

THE CONTROL OF BACTERIAL AND VIRUS DISEASES OF FISHES. S. F. Snieszko. p. 281. To conduct bioassays with a satisfactory level of reproducibility, it is necessary to have test fish of uniform quality. Parasitic, bacterial and viral diseases, malnutrition, and uncertain hereditary background can drastically affect the usefulness of the fish used for bioassays. Methods of control of bacterial and viral fish diseases with drugs such as sulfonamides and nitrofurans, with antibiotics such as chloramphenicol and tetracyclines, with chemical disinfectants, by immunization, by breed-

avec suffisamment de sureté, quant, à famille. Dès expériences de laboratoire sur la toxiquité de différents produits chimiques sur les larves *Trichoptera*, sont pratiquement inconnues. Cependant les résultats d'un groupe d'expérience fait à l'academie, sont présentés.

APPLICATION DE L'ÉCOLOGIE ET DE LA PHYSIOLOGIE DES DIATOMÉES AUX PROBLÈMES DE POLLUTION ETDE PURIFICATION DE L'EAU. F.E. Round. p. 29. On a étudié la distribution des diatomees dans plusieurs eaux polluées et aussi leur rapport avec les types observés dans des eaux naturelles non polluées. On y suggère un plan d'étude plus étendu des diatomées en eaux polluées et on y compare aussi les résultats obtenus par différentes méthodes d'évaluation de la pollution. La classification des milieux et les méthodes d'échantillonnage font le sujet d'une discussion. On y étudie en détails les exigences écologiques fondamentales des diatomées et leurs modifications en eaux polluées. On y indique aussi la manière probable d'agir des polluants sur les diatomées et les effets salutaires de la flore des diatomées sur la purification subséquente des eaux polluées. On y discute la mise en évidence de la pollution naissante d'après une étude des sédiments de lacs basée sur des espèces indicatrices et aussi le besoin de faire une nauvelle évaluation de ces espèces indicatrices.

LE CONTRÔLE DES MALADIES DES POISSONS PAR BACTERIES ET VIRUS. S. F. Snieszko. p. 281. Pour conduire ces tests avec un niveau satisfaisant de production, il est nécessaire de tester des poissons de qualité uniforme. Les maladies parasitiques, bactériologiques et virulentes, la malnutrition, la connaissance d'une incertaine hérédité peuvent affecter regoureusement l'entière utilité du poisson utilise pour les éssais biologiques. Les méthodes de contrôle des maladies de poissons par bacteries et virus avec des drogues telles que solfonamides, et nitrofurannes,

gesammelten Larven können mit genügender Sicherheit einer bestimmten Gattung zugeordnet werden, sodass Gattungsangaben nicht hinreichend zuverlässig sind. Laboratoriumsversuche bezüglich der Giftigkeit verschiedenerchemischer Stoffe gegenüber Eintagsfliegenlarven gibt es praktisch nicht; indessen werden die Ergebnisse einer einzigen Versuchsreihe, welche an der Akademie ausgeführt wurde, mitgeteilt.

DIE ANWENDUNG VON DIATOMEENOEKOLOGIE UND PHYSIOLOGIE AUF FRAGEN DER WASSERVERUNREINIGUNG UND REINIGUNG. Frank Eric Round. p. 29. Die Verteilung von Diatomeen über die verschiedenen verunreinigten Wasser und ihre Beziehungen zu Normen nicht verunreinigter natürlicher Wasser wird besprochen. Ein vorgeschlagenes weiteres Feld von Diatomeenstudien in verunreinigten Wassern durch Einschluss der Flora der einzelnen Vorkommen wird im Umriss beschrieben und die Ergebnisse mit experimentellen Methoden der Schätzung von Verunreinigung verglichen. Die Einteilung der Vorkommen, Probenahmeverfahren, grundlegende oekologische Erfordernisse der Diatomeen und deren Veränderung in verunreinigten Wassern werden besprochen. Die wahrscheinliche Form, in welcher die Verunreinigungen auf die Diatomeen einwirken und die vorteilhaften Auswirkungen der Diatomeenflora in der Selbstreinigung verschmutzter Wasser wird erörtert. Entdeckung beginnender Verunreinigung durch das Studium von Ablagerungen in Seen mittels Leitorganismen und die Notwendigkeit erneuter Auswertung von Leitorganismenarten werden vorgeschlagen.

DIE KONTROLLE VON BAKTERIEN - UND VIRUSERKRANKUNGEN VON FISCHEN. S. F. Snieszko. p. 281. Um biologische Versuche mit einem zufriedenstellenden Grad von Wiederholbarkeit durchzuführen, benötigt man Versuchsfische einheitlicher Güte. Parasiten -, Bakterien - und Viruserkrankungen, falsche Ernährung und ungewisse Angaben über Vererbung können die Nützlichkeit der Fische für biologische Versuche drastisch beeinflussen. Kontrollmethoden für Bakterien - und Viruserkrankungen der Fische mit Drogen wie Sulfanilamiden und Nitrofuranen, mit ing of disease resitant strains of fish, and by means of general sanitary measures are outlined.

EFFECTS OF SUBLETHAL CONCENTRATIONS OF ZINC AND COPPER ON MIGRATION OF ATLANTIC SALMON. J. B. Sprague. p. 332 . In 1960, the spawning migration of adult salmon in the northwest Miramichi River, New Brunswick, Canada, was disturbed. Of the salmon that moved upstream during the warm season, 22 percent came back downstream through a counting fence. In the previous six years, downstream movements had usually been less than 2 percent and had not exceeded 3 percent. The apparent cause was sublethal concentrations of zinc and copper in the river as a result of increased activity at the base-metal mine during 1960. Waste treatment at the mine in 1961 improved conditions somewhat, but downstream movement was still 14 percent. Disturbed migration occurred on days when pollution was high as happened during freshets.

Efforts to pinpoint the "safe" level of pollution for salmon migration were complicated by the presence of both zinc and copper, each varying somewhat independently of the other. Furthermore, it seemed likely that seasonal changes in water hardness would modify the "safe" level, similar to the way hardness changes lethal levels. A single index of effective pollution was obtained by using a system developed at the British Water Pollution Laboratory. From the water hardness in the Miramichi each day, the threshold lethal concentrations for zinc and for copper were estimated on the basis of laboratory work. Then for each day, the actual concentrations of zinc and copper in the Miramichi were expressed as fractions of their respective lethal thresholds. The two fractions for each day were added together yielding a single "toxicity index." Sharp variations of this index during 1961 corresponded well with disturbances of salmon migration. A "toxicity index" of 0.15 (15 percent of the threshold lethal concentration) seemed to be the maximum "safe" level for salmon migration. A

avec des antibiotiques tels que chloramphenical et tétracyclines, avec des désinfectants chimiques, par immunisation et par moyen de mesures sanitaires générales, sont ébauchées.

ÉFFETS DES CONCENTRATIONS NON FATALES DE ZINC ET DE CUIVRE SUR LA MIGRATION DU SAUMON DE L'ATLANTIQUE. J. B. Sprague. p. 332 . En 1960, la migration de ponte du saumon adulte a été dérangée dans la partie nord ouest de la rivièr Miramichi, Nouveau-Brunswick, Canada. A l'aide d'un barrage pour le comptage, nous avons observé que 22% des saumons qui avaient monte la rivière durant la saison chaude étaient revenus en aval. Durant les six annees précédentes, les déplacements avaient été ordinairement de l'ordre de 2% ou moins et jamais au dessus de 3%. Il semble que la cause de ce changement était des concentrations élevées mais non fatales de zinc et de cuivre dans la rivière, concentrations résultant d'une plus grande activité à la mine des métaux lourds en 1960. En 1961, le traitement des déchets à la mine a amélioré un peu les conditions, mais le déplacement en aval était encore de 14%. La migration a été dérangée durant les jours où la pollution était élevée, comme il arrivait durant la crue.

Les travaux pour déterminer le degré de pollution qui ne serait pas nuisible à la migration du saumon étaient compliqués par la présence de deux métaux, le zinc et le cuivre, dont les concentrations varient un peu indépendamment l'un de l'autre. De plus il semble vraisemblable que les changements saisonniers de la dureté de l'eau puissent modifier le niveau non nuisible comme d'ailleurs la dureté change les niveaux fatals. Un seul indice de pollution efficace a été obtenu en utilisant un système développé au Laboratoire des Recherches sur la pollution de l'eau, Angleterre.

A partir de la dureté de l'eau de la rivière Miramichi à chaque jour, on a pu estimer au laboratoire les seuils des concentrations fatales pour le zinc et pour le cuivre. Ensuite pour chaque jour, on a exprimé les concentrations actuelles de zinc et de cuivre dans

Antibiotika wie Chloramphenikol und Tetrazyklin, mit chemischen keimtötenden Mitteln, durch Immunisation, durch Züchten von gegen Krankheit widerstandsfähigen Arten von Fischen und durch allgemeine Gesundheitsmassnahmen werden dargelegt.

EINFLUSS VON SUBLETHALEN KONZENTRATIONEN VON ZINK UND KUPFER AUF DIE WANDERUNG DES ATLANTISCHEN LACHSES. J. B. Sprague. p. 332 . Im Jahre 1960 war die Laichwanderung des erwachsenen Lachses im nordwestlichen Miramichi - Fluss, New Brunswick, Canada unregelmässig. 22 % des Lachses, der während der warmen Jahreszeit stromaufwärts gewandert war, kam durch den Zählzaun stromabwärts zurück. In den vorausgegangenen sechs Jahren waren solche Stromabwärtswanderungen gewöhnlich unter 2 % und hatten nie 3 % überschritten. Anscheinend waren sublethale Konzentrationen von Zink und Kupfer im Fluss als Folge der vermehrten Tätigkeit eines Metallbergwerks während des Jahres 1960 die Ursache. Abwasserbehandlung am Bergwerk selbst während des Jahres 1961 besserte die Lage etwas, aber die Stromabwärtswanderung war immer noch 14 %. Die unregelmässige Wanderung fand an Tagen hoher Wasserverunreinigung, z. B. während Überschwemmungen, statt.

Die Bemühungen, eine „unschädliche" Höhe der Verunreinigung für Lachswanderung zu finden, waren dadurch erschwert, dass zwei Metalle vorhanden waren, deren Konzentrationen sich unabhängig voneinander änderten. Ausserdem schien es wahrscheinlich, dass zeitliche Änderungen der Wasserhärte die „unschädliche" Höhe beeinflussen würden, ähnlich wie die Härte die tödliche Konzentration ändert. Ein einziges Merkmal der tatsächlichen Verunreinigung wurde erhalten, wenn ein System, das am British Water Pollution Laboratory entwickelt worden war, benützt wurde.

Auf Grund der Wasserhärte im Miramichi - Fluss wurden täglich die Schwellenwerte der tödlichen Konzentrationen für Zink und für Kupfer im Laboratorium bestimmt. Sodann wurden für jeden Tag die im Miramichi gefundenen Konzentraconsistent correlation with salmon movements was not obtained if any of the three factors, zinc, copper, or water hardness, was ignored.

la rivière Miramichi comme fractions de leurs seuils fatals respectifs. Les deux fractions ont été additionnées pour chaque jour et on a alors obtenu un seul « indice de toxicité ». Des variations très marquées de cet indice en 1961, correspondaient bien avec les changements de la migration du saumon. Un « indice de toxicité » de 0.15 (15% du seuil des concentrations fatales) semble être le niveau maximum qui ne serait pas nuisible à la migration du saumon. On n'a pas pu obtenir de corrélation stable avec les mouvements du saumon, si l'un des trois facteurs, zinc, cuivre ou dureté de l'eau n'était pas considéré.

tionen von Zink und von Kupfer als Bruchteil der zugehörenden Schwellenwerte der tödlichen Konzentrationen ausgedrückt. Die beiden für jeden Tag errechneten Brüche wurden zusammengezählt und ergaben einen einzigen „Giftigkeitswert" . Grosse Veränderungen dieses Wertes während des Jahres 1961 stimmten gut mit den Unregelmässigkeiten der Lachswanderung überein. Ein „Giftigkeitswert" von 0,15 (15 % des Schwellenwertes der tödlichen Konzentration) schien der Höchstwert der „unschädlichen" Konzentration für Lachswanderung zu sein. Übereinstimmende Beziehungen mit den Wanderungen des Lachses konnten nicht erhalten werden, wenn einer der drei Faktoren, Zink, Kupfer oder Wasserhärte, unberücksichtigt blieben.

THE TAINTING OF FISH BY OUTBOARD MOTOR EXHAUST WASTES AS RELATED TO GAS AND OIL CONSUMPTION. Eugene W. Surber, John English, and Gerald McDermott. p. 170. Field studies to determine whether bluegills could be tainted under natural conditions were carried out. Three ponds were selected for this study. One of these, Oeder Lake, a 6.89-acre privately-owned pond, was popular to water skiing and boating enthusiasts. A record was kept of outboard motorboat usage in terms of gallons of fuel consumed. A second pond (the control) had an area of about 0.5 acres and was located in the same watershed, but did not recieve drainage from Oeder Lake. In a third pond (Eggerding Pond 0.96 acre) outboard motors were operated a known number of hours and fuel consumption recorded. Adult bluegills were placed in liveboxes in the three ponds and sampled periodically to determine whether, and how soon, their flesh became tainted. A 12-member panel was used to taste fish from each pond at intervals of about 3 weeks. The fish were prepared by two methods -- frying in vegetable oil with cracker crumbs at 380°F to brownness on both sides, and baking in aluminum foil at 350°F for 20 minutes. Cold milk was supplied each panel member to drink after testing each portion. The taste of each portion of fish was recorded on cards by three symbols: 0 = no objectionable taste; - = slightly objectionable taste; and + = strongly objectionable taste. The results were tested by Chi Square Analysis on the assumption that the fish in the control pond had equal opportunity to become tainted by natural or

ALTÉRATION DES POISSONS PAR LES EAUX USÉES DES HORS BORDS EN RELATION AVEC LA CONSOMMATION ENGAZETHUILE. Eugene W. Surber, John English and Gerald McDermott. p. 170. Des études sur le terrain furent enterprises pour déterminer si les bluegills peuvent être altérés dans des conditions naturelles. Trois étangs furent choisis pour ce travail, l'un d'eux, le Lac Oeder, étang privé de 6,89 acre (2,78 ha) était utilsé pour les sports nautiques (bateau, ski . . .). Un registre fut tenu pour l'usage des hors bords, exprimé en gallons de pétrole consommé. Un second étang de contrôle ayant une surface d'environ 0,5 acre (0,2 ha) était situé sur le même bassin versant mais ne recevait aucun écoulement de Lac Oeder. Dans un troisième étang (étang Eggerding de 0,96 acre (0,39 ha) les hors bord ne circulient que pendant un nombre d'heures déterminé et la consommation en pétrole était enregistrée. Des bluegills adultes furent placés dans des nasses, dans les trois étangs et des échantillons prélevés périodiquement pour déterminer si, et à partir de combien de temps, leur chair s'altérait. Un jury de 12 membres fut désigné pour gouter les poissons de chaque étang, toutes les trois semaines eviron. Les poissons étaient préparés de deux mainières: frits à l'huile végétale avec des miettes de biscuits (panés) à 380°F (193°C) jusquà brunissement sur chaque côté et cuits dans une feuille d'aluminium pendant 20 minutes à 350°F (176°C). Du lait froid était fourni à chaque membre après avoir gouté chaque portion. Le goût de poisson était indiqué par un symbole 0 - pas de gout désagréable, - = goût

DIE GESCHMACKSVERSCHLECHTERUNG VON FISCHFLEISCH DURCH DIE AUSPUFFGASE VON AUSSENBORDMOTOREN UND IHRE BEZIEHUNGEN ZUM KRAFTSTOFF - UND ÖLVERBRAUCH. Eugene W. Surber, John English, and Gerald McDermott. p. 170. Studien wurden unternommen, um festzustellen, ob eine Geschmacksverschlechterung des Fleisches von Bluegill *(Lepomis macrochirus macrochirus.* Rafinesque) unter naturlichen Bedingungen hervorgerufen werden könnte. Drei Weiher wurden zu diesem Zweck gewählt: der Oeder Lake von (6.89 acres) etwa 2,8 ha Fläche, Privateigentum und beliebt für Wasserski - und Bootsport. Der Kraftstoffverbrauch durch Aussenbordmotoren in U. S. gallonen (zu 3,79 l) wurde bestimmt. Ein zweiter Weiher von etwa (0.5 acres) 0,2 ha Fläche wurde zum Vergleich herangezogen; er lag im gleichen Einzugsgebiet, hatte aber keinen Zufluss vom Oeder Lake. In einem dritten Weiher, dem Eggerding Weiher von 0.96 acre oder etwa 0,4 ha Fläche wurden Aussenbordmotore eine bestimmte Anzahl von Stunden lang benützt und ihr Kraftstoffverbrauch gemessen. Erwachsene Bluegills wurden in den drei Weihern in Kästen ausgesetzt; in bestimmten Zeitabständen wurden Fische herausgenommen, um festzustellen, ob und wann ihr Fleisch eine Geschmacksveränderung erlitten hatte. Gruppen von 12 Personen unterwarfen Fische von jedem der drei Weiher in Zeitabständen von etwa 3 Wochen, jeweils einer Geschmacksprobe. Die Fische wurden auf zwei verschiedene Arten zubereitet: in Pflanzenöl mit Semmelbröseln bei 380° F oder etwa

other causes. The significance of Chi Square values could have been reduced by decomposition products of algal or vegetation decay, pollution from the few ducks inhabiting the pond, etc., but the data showed these factors to be unimportant.

When the over-all season's results were analyzed for livebox fish from Oeder Lake, combining slightly objectionable and strongly objectionable as objectionable in both Oeder Lake and the Control Pond, a significant difference, Chi Square 4.425, was observed for fish fried in vegetable oil and cracker crumbs (Chi Square at probability 0.05 for one degree of freedom = 3.841). For those baked in aluminum foil, the significance was even greater, 12.74. In Eggerding Pond, where outboard motors were operated a known length of time, fish became tainted within a month. An outbreak of *Cytophaga columnaris* then killed all livebox fish, but tasting of other fish outside the boxes was carried out, as well as later tasting of livebox-confined fish, which again became tainted within a month. When the Chi Square test for significance was applied to all portions of bluegills fried from the beginning of panel sessions with Eggerding Pond bluegills, a high value of 20.8 was obtained. Baking produced even more striking results with a high Chi Square value of 22.67.

légèrement désagréable et + = gout très désagréable.

Les résultats furent analysés au test (X^2) avec l'hypothèse que les poissons de l'étang de contrôle pouvaient également être altérés par des causes naturelles ou autres. La signification des valeure de X^2 auraient pu être réduite par des produits de décomposition des algues ou des vegétaux par une pollution due aux canards habitants l'étang etc . . . . mais les résultats ont montré que ces facteurs n'étaient pas importants.

Quand les résultats de toute la saison furent analysés pour les poissons du Lac Oeder en combinant les « légèrement désagréables » et les « très désagréables » en un seul groupe « désagréable » à la fois pour le lac Oeder et pour l'étang de contrôles, une différence significative (X^2 = 4,425) fut observée pour les poissons panés (X^2 pour 1 degré de liberté et une probabilité de 0,05 = 3,841). Pour ceux cuits dans l'aluminium, la signification était encore plus grande : 12,74. Dans l'étang d'Eggerding où les hors bord ne circulaient que pendant des temps donnés, les poissons furent altérés en 1 mois. Un développement brutal de Cytophaga columnaris tuait alors tous les poissons dans les nasses mais la dégustation d'autres poissons, extérieurs aux nasses, fut poursuivie ainsi que celle de nouveaux poissons enfermés qui, cette fois encore, s'altérèrent en un mois. Quand le test X^2 fut appliqué à toutes les portions de poissons frits depuis le début des expériences avec les bluegills de l'étand d'Eggerding, la valeur élevée de 20,8 fut obtenue. La cuisson dans l'aluminium donnait des résultats encore plus frappants avec la valeur de X^2 - 22.67.

195° C in der Pfanne auf beiden Seiten gebraten, oder 20 Minuten lang in Aluminiumfolie eingeschlagen bei 350° F oder etwa 175° C gebacken. Den Versuchsteilnehmern wurde nach jeder Geschmacksprobe kalte Milch zum Trinken vorgesetzt. Der Geschmack jeder Fischprobe wurde auf Karten durch 3 verschiedene Zeichen ausgedrückt; 0 = einwandfrei, - = nicht ganz einwandfrei, und + = stark unerwünschter Geschmack. Die Ergebnisse wurden durch die X^2 Analyse geprüft unter der Annahme, dass Geschmacksveränderungen des Fischfleisches im Vergleichsweiher durch natürliche oder andere Ursachen ebenso möglich gewesen wären wie in den anderen zwei Weihern. Die Bedeutung der X^2 Werte hätte durch Zersetzungsstoffe von Algen oder anderen pflanzlichen Stoffen, durch Verunreinigung durch einige Enten in dem Weiher usw. herabgesetzt werden können; die Ergebnisse zeigten indessen, dass diese Einflüsse unbedeutend waren.

Für die Analyse der Ergebnisse einer Versuchsreihe an Fischen aus den Kästen vom Oeder Lake wurden die - und die + Werte dieses Weihers und des Vergleichsweihers als nicht einwandfrei zusammengefasst und ergaben einen deutlichen Unterschied, X^2 = 4,425, für die in Pflanzenöl mit Semmelbröseln gebratenen Fische (X^2 = 3,841 für einen Freiheitsgrad bei Wahrscheinlichkeit 0,05). Für die in Aluminiumfolie gebackenen Fische war der Unterschied sogar noch grösser, nämlich 12,74. Im Eggerdingweiher, wo Aussenbordmotore eine bekannte Zeitlang benützt wurden, war eine Geschmacksverschlechterung des Fischfleisches innerhalb eines Monats eingetreten. Ein Ausbruch von *Cytophaga columnaris* tötete dann alle Fische in den Kästen, jedoch wurden die freischwimmenden Fische der Geschmacksprobe unterworfen; später wurden wiederum in Kästen gehaltene Fische geprüft: sie waren ebenfalls nach einem Monat wieder nicht einwandfrei. Wenn die X^2 Probe auf alle Proben von Bluegills angewandt wurde, die vom Versuchsbeginn an dem Eggerdingweiher entnommen worden waren, dann erhielt man einen hohen Wert von 20,8. Backen gab sogar den viel höheren Wert von 22,67.

EUTROPHICATION OF LAKES AND RIVERS. E. A. Thomas. p. 299. A plentiful supply of fertilizers reaches algae and higher aquatic plants in rivers and lakes by means of sewage, even when the sewage is mechanically and biologically purified. Nitrates and phosphates, which are particularly important as fertilizers, stimulate the growth of algae and higher aquatic plants; this leads to manifold damages and complications. The direct measure against the eutrophication of rivers and lakes that promises the greatest success is the thorough removal of phosphates from the sewage. If the sewage from industrial plants is rich in phosphates, the phosphates should be removed, where possible, within the plant, or such sewage should be used for agricultural purposes. Reference is made to some further aids to prevent eutrophication of lakes and rivers. The special limnological character of each type of sewage must be especially considered when choosing preventive measures.

EUTROPHICATIONS DES LACS ET RIVIERÈS. E. A. Thomas. p. 299. Une alimentation complète de fertilisants s'étends aux algues et hautes plantes aquatiques en fleuves et lacs, au moyen d'égouttage, même quand celui-ci est mécaniquement et biologiquement purifié. Les nitrates et phosphates qui sont particuliérement importants comme engrais stimule la croissance des algues et des haute plantes aquatiques.

La mesure directe contre l'eutrophication des rivières et lacs qui promet le plus grand succès est l'élimination complète des phosphates des eaux d'égout. Si l'égout provenant d'usines industrielles est riche en phosphate, le phosphate doit être éliminé, si possible dans l'usine même ou de tels déchets seraient utilisés à des fins agricoles. La recommendation est faite pour d'autres aides additives afin de prévenir l'eutrophication des rivières et lacs. Le caractère speciale limnologique de chaque type d'eau d'egout, doit être spécialement considéré quand des mesures préventives sont prises.

EUTROPHIKATION VON SEEN UND FLÜSSEN. E. A. Thomas. p. 299. Algen und höhere Wasserpflanzen in Flüssen und Seen erhalten eine reiche Zufuhr von Düngemitteln durch Abwässer, auch wenn diese mechanisch und biologisch gereinigt wurden. Nitrate und Phosphate, welche als Düngemittel besonders wichtig sind, reizen das Wachstum von Algen und höheren Wasserpflanzen; diese Tatsache führt zu mannigfachen Schädigungen und Schwierigkeiten. Die unmittelbare Massnahme gegen diese Eutrophikation von Flüssen und Seen, welche den grössten Erfolg verspricht, ist die gründliche Entfernung von Phosphaten aus den Abwässern. Wenn die Abwässer von industriellen Unternehmen phosphatreich sind, dann sollten die Phosphate möglichst in der Fabrik selbst entfernt werden oder die Abwässer sollten für landwirtschaftliche Zwecke verwendet werden. Einige weitere Abhilfen gegen die Eutrophikation von Seen und Flüssen werden erwähnt. Der limnologische Charakter des Abwassers muss jeweils besonders in Betracht gezogen werden, wenn Vorbeugungsmassnahmen gewählt werden.

EFFECTS OF COOLING WATER FROM STEAM-ELECTRIC POWER PLANTS ON STREAM BIOTA. F.J. Trembley. p. 334. The generation of electricity by steam-electric stations requires the use of large amounts of water as a coolant. This necessitates the release of heated water into rivers, lakes, and estuaries. The demand for electric power in the United States is increasing by about 10 percent annually. Thus the problem of release of heated water is rapidly becoming acute. Laws and regulations concerning such releases are often vague and unrealistic or in many states are non-existent. A brief account of research done in Pennsylvania, and observations made in England are given. Suggestions are made for planning research in this field, and an improved communication system for research findings is suggested.

EFFETS DE L'EAU DE RÉFRIGÉRATION DE CENTRALES THERMIQUES SUR LES COURANTS D'EAU. F. J. Trembley. p.334. La génération de l'électricité par centrales thermiques nécéssite, comme réfrigérant, une grande quantité d'eau. Ceci nécéssite par conséquent, le rejet, de l'eau chaude utilisée dans les fleuves, lacs et estuaires. La demande en électricité aux USA s'accroît généralement de dix pour cent par an. Aussi le problème du rejet de l'eau chaude est rapidement venu aigu. Les lois concernant de tels rejets sont souvent vagues et inréalistes ou dans certain états, non existants. De brèves recherches en Pennsylvania et des observations faites en Angleterre ont été donnés. Des suggestions sont faites pour planifier les recherches dans ce domaine et un système de communication pour des recherches ayant abouti, est suggéré.

WIRKUNGEN DES KÜHLWASSERS VON DAMPFKRAFTWERKEN AUF DIE LEBEWELT IN FLÜSSEN. F. J. Trembley. p. 334. Die Erzeugung elektrischer Energie durch Dampfkraftwerke erfordert grosse Kühlwassermengen und diese wiederum verursacht das Einleiten von warmem Wasser in Flüsse, Seen und Flussmündungen. Die Nachfrage nach elektrischer Energie nimmt in den Vereinigten Staaten jährlich um etwa 10 % zu, sodass das Problem der Beseitigung des warmen Wassers rasch kritisch wird. Die Gesetze und Vorschriften, die sich mit solchen Entleerungen befassen, sind oft unklar und wirklichkeitsfremd oder in manchen Staaten überhaupt nicht vorhanden. Ein kurzer Bericht über Studien in Pennsylvanien und Beobachtungen in England werden gegeben. Anregungen zur Planung von Forschungsarbeiten auf diesem Gebiet werden gemacht und ein verbesserter Plan zur Mitteilung der Forschungsergebnisse wird vorgeschlagen.

TOXICITY OF SOME HERBICIDES, INSECTICIDES, AND INDUSTRIAL WASTES. P. Vivier and M. Nisbet. p. 167 . Toxicity of herbicides: In the laboratory as well as in the field, our bioassays with Simazine and Atrazine have shown that these products are greatly toxic when in water, but are harmless to certain plants that immediately absorb them. Weedex, Weedazol, etc., are generally less injurious, even at concentrations much higher than the normal dose employed.

Toxicity of copper sulfate: Long-term studies of a small stream by L. Mazoit, et al., showed that in 6 days 75 percent of the carp and tench are killed by a 0.2 ppm concentration of copper. Most of the minnows are also killed, but some of them can tolerate 0.3 ppm. In the nutritive fauna, larvae of Ephemeropterae are killed, but those of Trichopterae live but seem to be excited. The water of this stream was hard.

Toxicity of synthetic detergents: The studies of J. Wurtz-Arlet have shown that the anionic and non-ionic detergents are toxic for the eggs and young brown trout *(Salmo fario)* and young rainbow trout *(Salmo gairdneri)*. The 24-hour TL_m is 3 to 5 ppm for the rainbow trout, depending on the age of the fishes; these quantities are lethal for the young brown trout, however, in less than 10 hours.

Toxicity of sodium chloride: Bioassays were performed by P. Laurent on numerous aquatic animals with a sodium chloride solution of 10,000 ppm. The cyprinids resisted for 10 to 60 days, according to the temperature. The nutritive faunas *(Asellus, Hydropsyche, Dreissensia, Sphaerium)* are more sensitive; 50 percent were decimated in from 2 to 6 days, as were also the most current plants found in the stream *(Gallitriche, Melosciadium*, and *Oenanthe)*.

TOXICITÉ DE QUELQUES HERBICIDES, INSECTICIDES ET EAUX INDUSTRIELLES. P. Vivier and M. Nisbet. p. 167. Toxicité d'herbicides: au laboratoire aussi bien que sur le terrain, nos tests avec la Simazine et l'Atrazine ont montré que ces produits étaient très toxiques à l'état dissous dans l'eau, mais beaucoup moins dangereux s'il y a des plantes supérieures qui les absorbent rapidement. Weedex, Weedazol, etc . . . sont généralement moins nocifs, même à des concentrations très supérieures aux doses normales d'emploi.

Toxicité du Sulfate de Cuivre: des études à long terme effectuées dan un petit ruisseau par L. MAZOIT et Coll. ont montré qu'en 6 jours, 75% des carpes et tanches étaient tuées par une concentration de 0,2 ppm de cuivre. La plupart des vairons étaient tués également, mais quelques uns toléraient 0,3 ppm. Parmi la faune nutritive, les larves d'Ephemeroptera étaient tuées, mais celles de Trichoptera résistaient et semblaient excitées. L'eau de ce ruisseau était dure.

Toxicité des détergents synthétiques: les études de J. WURTZ-ARLET ont montré que les détergents anioniques et non-ioniques sont toxiques pour les oeufs et les alevins des truites fario *(Salmo fario)* et les alevins de truites arc-en-ciel *(Salmo gairdneri)*. La concentration correspondante à une tolérance limite moyenne de 24 h. est de 3 à 5 ppm pour la truite arc-en-ciel selon l'âge des poissons; ces quantités sont toutefois mortelles pour les alevins de truite fario en moins de 10 heures.

Toxicité du Chlorure de Sodium: des tests à long terme furent poursuivi par P. LAURENT sur de nombreaux animaux aquatiques avec une solution de chlorure de sodium à 10.000 ppm. Les cyprinidés résistaient de 10 à 60 jours selon la température. La faune nutritive *(Asellus, Hydropsyche, Dreissensia, Sphaerium)* est plus sensible: 50% disparaissent entre 2 et 6 jours ainsi que les plantes les plus communes des ruisseaux *(Gallitriche, Melosciadium, Oenanthe)*.

GIFTIGKEIT EINIGER UNKRAUTVERTILGUNGSMITTEL, INSEKTENVERTILGUNGSMITTEL UND INDUSTRIEABWASSER. Paul Vivier et Maud J. Nisbet. p. 167. Giftigkeit von Unkrautvertilgungsmitteln: Im Laboratorium und auf dem Versuchsfeld haben unsere biologischen Versuche mit Simazin [(2 - Chlor-4, 6-bis (äthylamino) - 1,3,5 - Triazin] und mit Atrazin (2 - Chlor - 4 - äthylamino - 6 - isopropylamino - 1,3,5 - Triazin) gezeigt, dass diese Stoffe in wässriger Lösung sehr giftig, aber für gewisse Pflanzen, welche sie unmittelbar absorbieren, unschädlich sind. Weedex, Weedazol, usw. sind im allgemeinen weniger schädlich, sogar wenn man sie in viel höheren Konzentrationen anwendet als der normalen Menge entsprechen würde.

Giftigkeit von Kupfersulfat: Studien von L. Mazoit und Mitarbeitern über lange Versuchszeiten hin an einem kleinen Fluss zeigten, dass in 6 Tagen 75% der Karpfen und Schleien durch 0,2 mg/l Kupfer eingegangen waren. Auch die meisten Elritzen waren tot, aber einige konnten 0,3 mg/l ertragen. In der Nahrungsfauna wurden Larven von Ephemeroptera getötet, die von Trichoptera hingegen nicht; sie schienen jedoch erregt zu sein. Das Wasser dieses Flusses war hart.

Giftigkeit synthetischer Weichmachungsmittel: Studien von J. Wurtz - Arlet zeigten, dass anionische und nicht ionisierte Weichmachungsmittel für die Eier und für junge braune Forellen *(Salmo fario)* und für junge Regenbogenforellen *(Salmo gairdnerii)* giftig sind. Die Werte für TL_m für 24 Stunden sind für Regenbogenforellen 3 bis 5 mg/l, wobei das Alter der Fische eine Rolle spielt; die gleichen Mengen sind aber für junge braune Forellen bereits in weniger als 10 Stunden tödlich.

Giftigkeit von Natriumchlorid: P. Laurent benützte in biologischen Versuchen mit zahlreichen Wassertieren eine Natriumchloridlösung von 10 Gramm im Liter. Cypriniden lebten darin 10 bis 60 Tage lang je nach der Versuchstemperatur. Die Nahrungsfauna *(Asellus, Hydropsyche, Dreissensia, Spaerium)* ist empfindlicher: 50 % gingen in 2 bis 6 Tagen ein. Die meisten Pflanzen im Versuchsgewässer *(Gallitriche, Melosciadium* und *Oenanthe)* wurden ähnlich betroffen.

THE VALUE AND USE OF WATER QUALITY CRITERIA TO PROTECT AQUATIC LIFE. A RATIONAL INDUSTRIAL VIEWPOINT. Roy F. Weston. p. 9 . No one individual can hope to represent the many diversified philosophies and opinions of industrialists on the subject of water quality criteria. Factors common to most industrial operations make it possible, however, to rationalize an industrial viewpoint.

Water quality criteria for aquatic life cannot be considered alone. They must be considered as a specific part of a broad spectrum of criteria pertaining to numerous different water uses.

It is axiomatic that water quality criteria for the protection of aquatic life are essential to an effective water pollution control program.

Philosophical concept confuses the establishment, application, and enforcement of such criteria.

The industrialist's policies must be controlled by his inherent philosophies, his training, his experience, and his responsibilities; i.e., (the hard facts of economic realism). Consequently, he must have sound and logical reasons for expending funds on water pollution control. He cannot rationalize the expenditure of funds in excess of that which is essential to equity or to the protection of a stream's best use. Once this has been established, he has reason to expect specific and reasonable water quality criteria. He should be required to meet only those criteria that the regulatory agency is prepared to monitor and to enforce.

The establishment and enforcement of reasonable technical and administrative policies and procedures present one of the greatest challenges to the pollution control profession. The success or failure of pollution control programs depends on the soundness of the regulatory agency's actions.

VALEUR ET EMPLOI DES CRITERES DE QUALITE DES EAUX POUR PROTEGER LA VIE AQUATIQUE UN POINT DE VUE INDUSTRIEL RATIONNEL. Roy F. Weston. p. 9 . Aucun individu ne peut espérer représenter les philosophies et opinions si diverses des industriels au sujet des critères de qualité des eaux. Les facteurs communs à la plupart des opérations industrielles rendent toutefois possible la rationalisation d'un point de vue industriel.

Les critères de qualité pour la vie aquatique ne peuvent être considérés seuls, mais comme une partie spécifique d'un ensemble de critères concernant les nombreux emplois de l'eau.

Il est évident que des critères de qualité des eaux pour la protection de la vie aquatique sont essentiels pour établir un programme de contrôle des pollutions.

Le concept philosophique confond l'établissement, l'application et l'exécution de tels critères.

Les lignes de conduite d'une industriel doivent être contrôlées par ses philosophies naturelles, son éducation, son expérience et ses responsabilités, c'est-à-dire les dures réalités d'une réalisme économique. En conséquence, il doit avoir des raisons solides et logiques pour dépenser des capitaux pour le contrôle de la pollution. Il ne peut justifier une dépense supérieure à celle nécessaire à l'équité au à la protection du meilleur emploi d'un cours d'eau. Une fois ceci établit, il a raison d'attendre des critères de qualité, spécifiques et raisonnables. On ne devrait exiger de lui que les critères pouvant être contrôlés et appliqués par le bureau régulateur.

L'établissement et l'application de directives techniques et administratives raisonnables présentent un des plus grands défis à la profession de contrôle des pollutions. Le succès ou l'échec des programmes de contrôle dépend de la justesse des actions du bureau régulateur.

WERT UND ANWENDUNG VON WASSERGÜTENORMEN ZUM SCHUTZ DES LEBENS IM WASSER, EIN RATIONALER INDUSTRIESTANDPUNKT. Roy F. Weston. p. 9. Niemand kann hoffen, der er persönlich die vielen verschiedenartigen Philosophien und Meinungen der Männer der Industrie hinsichtlich Wassergütenormen typisch darstelle. Es gibt indessen allen Industrieen eigene Tätigkeiten, die es ermöglichen, einen industriellen Standpunkt herauszuarbeiten.

Wassergüteeigenschaften können nicht für sich allein besprochen werden, da sie ein ganz bestimmter Teil des weiten Feldes der zahlreichen verschiedenen Anwendungen des Wassers sind.

Es wird allgemein anerkannt, dass Wassergütenormen zum Schutz des Lebens im Wasser für ein wirksames Programm zur Kontrolle der Wasserverunreinigungen wichtig sind.

Philosophische Begriffe verwirren die Festsetzung, Anwendung und Durchsetzung solcher Normen.

Die Massnahmen des Mannes der Industrie müssen durch seine angeborenen Philosophien, seine Schulung, seine Erfahrung und durch seine Verantwortlichkeiten, also durch die harten Tatsachen wirtschaftlicher Notwendigkeiten gezügelt werden.

Folglich muss er gute und logische Gründe für Geldaufwendungen für Wasserverunreinigungskontrolle haben. Er kann sich nicht mit solchen Ausgaben abfinden, welche höher sind als was zur Sicherung der besten Verwendung des Flusses recht und billig wäre. Sowie diese Verwendung festgelegt ist, hat er Grund, ins Einzelne gehende und wirklichkeitsnahe Wassergütenormen zu erwarten. Er sollte nur an die Beachtung solcher Normen gebunden sein, welche die Überwachungsbehörde ernstlich anzuwenden und unparteiisch durchzusetzen gewillt ist.

Die Festsetzung und Einhaltung vernünftiger technischer und Verwaltungsmassnahmen und Verfahren stellt eine der grössten Herausforderungen für die Männer dar, welche beruflich mit der Verunreinigungskontrolle zu tun haben. Der Erfolg oder Misserfolg der Verunreinigungskontrollprogramme hängt von der absoluten Vertrauenswürdigkeit der Überwachungsbehörde und ihren Massnahmen ab.

BIOLOGY OF WATER TOXICANTS IN SUBLETHAL CONCENTRATIONS. Charles G. Wilber. p. 326 . In evaluating the biological effects and significance of water toxicants the real importance of time must not be underestimated. All persons working with problems of water pollution recognize the significance of concentration of an agent. Time, including exposure time and latent period, is a real factor that modifies one's estimation of the biological activity of a given toxicant. The following general equation is discussed and evaluated:

$$C t = K + a t^{b + 1}$$

where C is concentration of toxicant, t the exposure time, K a constant related to species susceptibility, a is a constant related o magnitude of rate of detoxication, and b a constant related to change in rate of detoxication. The danger of using the LD_{50} concept for work nvolving animals that carry an aquatic respiration is illustrated. For critical biological assay of water toxicants, the percent survival of experimental species at a given ime gives sparse information. A more informative program is one nvolving graded response; in many nstances such a program will require time periods equal to the total ength of life of the test organism. n long-term exposures the logarithmic nature of biological time seems to be illustrated; the atending problems of this apparent phenomenon are discussed. Such ong exposure times with accompanying low concentration of oxicant are essential, if carcinogenic materials are under test with he view to extrapolation to potential human hazard. In our laboratory we consider essential (and are studyng) the effect of long-term exosures on organ weight-body weight atios; changes in these ratios are, in many instances, delicate indiators of subthreshold effects of oxicants. Examples from the literture and from our own data are resented to illustrate various points. The question, "Is there any scientifically acceptable upper mit for carcinogenic pollutants in water destined for human use?" is touched upon. The obvious requirements for adequate histopathogical and histochemical studies of ssues from test specimens is remphasized.

BIOLOGIE DES TOXIQUES DE L'EAU EN CONCENTRATIONS SUBLÉTHALES. Charles C. Wilber. p. 326 . En évaluent les effets biologiques et la signification des toxiques dans l'eau, l'importance réelle du temps ne doit pas être sous-estimée. Tous ceux qui travaillent sur les problèmes de pollution reconnaissent l'importance de la concentration d'un produit. La durée, comprenant le temps d'exposition et la période de latence, est un facteur réel qui modifie l'estimation de l'activité biologique d'un toxique donné. L'équation générale suivante est discutée et calculée:

$$C t = K + a t^{b + 1}$$

où C est la concentration du toxique, t le temps d'exposition, K une constante liée à la sensibilité des espèces, a une constante liée à la vitesse de désintoxication et b une constante liée au changement de la vitesse de désintoxication. Le danger de l'emploi de la LD_{50}, concept de travail pour des animaux qui continuent à respirer, est démontrée. Pour les essais biologiques, le pourcentage de survivants des espèces expérimentales à un moment donné, donne peu d'information. On obtient plus de renseignements avec des expériences donnant une résponse progressive; dans bien des cas de telles expériences nécessiteront des durées égales à la durée de vie de l'organisme testé. Dans les essais à long terme, la nature logarithmique due temps biologique semble être illustree; les problèmes en relation avec ce phénomène sont discutés. Les longues durées pour de faibles concentrations de toxiques sont indispensables s'il s'agit de tests effectués avec des produits carcinogènes en vue de l'extrapolation aux risques humains. Dans notre laboratoire, nous considérons comme essentielle l'action des longues exposition sur les rapports poids des organes - poids du crops; des changements dans ces rapports sont dans bien des cas de précieux indicateurs de seuils d'action des toxiques.

Des exemples choisis dans la littérature et dans nos propres expériences sont présentés pour illustrer divers points. Nous abordons la question Y a-t-il une limite acceptable scientifiquement pour les toxiques carcinogènes dans l'eau destinée à l'alimentation humane? Nous soulignons à nouveau la nécessité des études histopathologiques et histochimiques des tissues sur les spécimens d'expérience.

DIE BIOLOGIE VON GIFTSTOFFEN IM WASSER IN SUBLETHALEN MENGEN. Charles G. Wilbur. p.326 Für die Amswertung biologischer Wirkungen und die Bedeutung von Giftstoffen im Wasser darf die Wichtigkeit der Zeit nicht unterschätzt werden. Wer mit Problemen der Wasserverunreinigung zu tun hat, sieht die Bedeutung der Konzentration eines Stoffes ein. Die Zeit, einschliesslich der unmittelbaren Einwirkungszeit und der Latenzzeitspanne, ändert die Bestimmung der biologischen Wirksamkeit einer gegebenen Giftsubstanz oft sehr erheblich. Die folgende allgemeine Gleichung

$$C \quad t = K + a \quad t^{b} \; ^{+1}$$

wird besprochen und ausgewertet. In ihr ist C die Konzentration des Giftstoffes, t die Einwirkungszeit, K ein Festwert im Zusammenhang mit der Empfindlichkeit der Art, a ein Festwert, der mit der Grösse der Entgiftungsgeschwindigkeit und b ein Festwert, der mit der Änderung der Entgiftungsgeschwindigkeit zusammenhängt. Die Gefahren in der Anwendung des LD^{50} Begriffes für Arbeiten mit im Wasser atmenden Tieren wird an Beispielen gezeigt. Für die kritische biologische Prüfung von Giftstoffen gibt der Hundertsatz des Überlebens einer Versuchsart zu einer bestimmten Zeit nur spärliche Auskünfte. Lehrreicher ist ein Programm mit gestuften Ergebnissen; ein solches erfordert in vielen Fällen Versuchszeiten, die gleich der Lebensdauer der Versuchsorganismen sind. Dauerversuche deuten auf die logarithmische Natur der biologischen Zeit hin; die diesen möglichen Zusammenhang begleitenden Fragen werden erörtert. Lange Versuchszeiten und die damit verbundenen niedrigen Konzentrationen der Giftstoffe sind besonders wichtig, wenn krebserregende Stoffe in der Absicht geprüft werden, aus den Ergebnissen Schlüsse auf die möglichen Gefahren für den Menschen zu ziehen. In den Dauerversuchen, welche in unserem Laboratorium im Gange sind, halten wir die Feststellung der Verhältnisse Organgewicht/Körpergewicht für notwendig; Änderungen in diesen Verhältnissen sind in vielen Fällen empfindliche Nachweise von Giftwirkungen, die gerade noch unter dem Schwellenwert der betreffenden Gifte liegen. Zur Erläuterung der angeschnittenen Fragen werden Beispiele aus der Literatur und aus eigenen Versuchsreihen mitgeteilt. Die Frage „Besteht eine wissenschaftlich annehmbare obere Grenze für krebserregende Verunreinigungen in Wasser, das für menschlichen Genuss und Gebrauch vorgesehen ist?" wird aufgeworfen. Die klare Notwendigkeit für hinreichende histopathologische und histochemische Studien an Gewebeprobestücken wird wiederum betont.

SOME RESULTS OF OYSTER-PULP MILL WASTE BIOASSAYS. Charles E. Woelke. p. 67 . Five general types of bioassays of sulphite waste liquor with oysters, carried out by the Washington State Department of Fisheries, are described. These include field live boxes in waters receiving sulphite waste liquor, long range laboratory bioassays, and finally, 48-hour bioassays of field water brought to the laboratory. Based on the response of adult Olympia oysters *(Ostrea lurida)* in field live boxes and in long range bioassays, of adult Pacific oysters *(Crassostrea gigas)* in lagoon studies, and of the larvae of both species in short-term laboratory and field water bioassays, it is concluded that the fertilization and embryonic development processes of both species are affected at lower concentrations of sulphite waste liquor than the concentrations that affect the adults. To determine whether the toxicity of sulphite waste liquor present in the receiving waters differs from that of dilutions prepared in the laboratory, larval responses at the same levels are compared.

DES RÉSULTATS SUR LES HUÎTRES Charles E. Woelke. p. 67 . Cinq types généraux de tests de pertes de liqueurs sulphites relative aux huîtres qui ont été mis a exécution par le départment des pêcheries d'état de Washington, sont décrits. Ceux-ci incluent les parcs dans les eaux recevant ces pertes de liqueur sulfite, une grande étendue de test en laboratoire et finalement 48 heures de tests d'eau de terrain apportée au laboratoire. Basé sur le test de réaction de l'huître adulte Olympia *(Ostrea lurida)* en parc et de longue durée et de l'huître adulte Pacific *(Crassostrea gigas)* etudié en lagune, et des larves des deux especes étudiés en laboratoire et en eau de terrain, il est conclu que la fertalisation et le procédé de développement embryonnaire sont affectés au plus basses concentrations de liqueur sulphite perdues que les concentrations qui affectent les adultes. La détermainaison de la toxiquité de la liqueur sulphite perdue dans les eaux diffère des dilutions préparées en laboratoire. Les réactions des larves au mêmetaux sont comparées.

EINIGE BIOVERSUCHSERGEBNISSE MIT AUSTERN IN ZELLSTOFFABRIKABWÄSSERN. Charles E. Woelke. p. 67 . Es werden fünf Bioanalysenmethoden zur Untersuchung von Zellstoffabrikabwässern mit Austern, wie sie am Washington State Department of Fisheries durchgeführt wurden, beschrieben, darunter die Anwendung von Kästen in Gewässern, welche die Zellstoffabrikabwässer führten, Laboratoriumsdauerversuche mit lebendem Material und schliesslich 48-Stunden-Bioversuche an Flusswässern im Laboratorium. Aus dem Verhalten erwachsener Olympiaaustern *(Ostrea lurida)* in Kasten an Ort und Stelle und in Dauerversuchen, mit Pazifischen Austern *(Crassostrea gigas)* in Lagunenstudien und mit den Larven beider Arten in kurzfristigen Laboratoriumsversuchen und Bioversuchen an Ort und Stelle wird geschlossen, dass die Befruchtung und die Embryonalentwicklungsprozesse beider Arten durch geringere Konzentrationen von Zellstoffabrikabwässern beeinflusst werden als erwachsene Tiere. Zwecks Feststellung, ob die Giftigkeit der Zellstoffabrikabwässer in den Vorflutern von jener verdünnter Laboratoriumslösungen verschieden ist, wurden die Reaktionen von Larven unter identischen Konzentrationsverhältnissen verglichen.

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